INTRACELLULAR CILIATES AND HAEMOCYCTIC NEOPLASIA DYNAMICS IN DIGESTIVE GLAND OF MEDITERRANEAN MUSSELS

(Mytilus galloprovincialis)

IN SLOVENE SEA IN CORRELATION WITH SEA TEMPERATURE, SALINITY AND OXYGENATION

Mitja Gombač
The Slovene Sea

- part of the Gulf of Trieste, the northernmost end of the Adriatic Sea
- average temperature: 15.8 °C
- average salinity: 37 ppt
- average oxygenation: 7.5 mg O₂/l
- the deepest point: 37 m
- constant inflow of sweet water
MUSSELS CULTURES

Debeli rtič

Seča

Strunjan

Foto: Sergio Gobbo
only Mediterranean mussels (Mytilus galloprovincialis) are bred
seeds of 1 to 1.5 cm shell length are collected from natural beds (buoys, ropes, “seeds trap” etc.) in autumn
seeds are filled in cotton socks, which are put in nylon sleeves
sleeves are fastened on ropes, which hang from rafts
after 6 months mussels are removed, cleaned and put in bigger socks
mussels are once or twice exposed to direct sunlight for a whole day
they rich market size in 18 months (5 to 7 cm length)
INTRACELLULAR CILIATES OF MUSSELS

- *Rhynchodid*-like ciliates (pear or spindle-shaped, 3.9-15 μm),
- presumably ubiquitous protozoans, belonging to the phylum *Ciliophora*,
- inhabit digestive glands,
- unknown way, condition and transmission of infection,
- the prevalence of infection: 1–40%?
- harmless commensals?
HAEMOCYTIC NEOPLASIA OF MUSSELS

- the proliferation of large neoplastic haemocytes (12 – 30 μm),
- unknown aetiology,
- ubiquitous disease,
- unknown way, condition and transmission of disease,
- the prevalence: only sporadic cases,
- pathology: neoplastic haemocytes replace normal ones, tissue degeneration, necroses, death.
MATERIAL AND METHODS

- **Sampling**
  - 80 cultured and 40 wild Mediterranean mussels were monthly sampled throughout one year period.
  - Water temperature, salinity and oxygenation were measured at each sampling.

- **Measurements and the calculation of condition index**
  - Measurement of the length and the total weight of each mussel;
  - Weighting of mussel flesh;
  - Calculation of the condition index on fresh mussels using formula: “condition index = fresh flesh weight x 100/total weight”
- **Macroscopic examination**
  - mussels were macroscopically inspected for eventual abnormalities or lesions.

- **Histological examination**
  - a standard section through the visceral mass was performed according which one sample from each mussel was taken, put in 10% formalin solution for 24 hours, embedded in paraffin and cut in 4 μm sections, stained with haematoxylin and eosin and examined by light microscopy for the presence of intracellular ciliates and neoplasias.
The evaluation of the intensity of the infection with intracellular ciliates
- uninfected: no parasites detected;
- mild infection – less than 50 parasites per section;
- moderate infection – from 50 to 100 parasites per section;
- severe infection – more than 100 parasites per section.

Statistical analyses
- Testing of the hypotheses required a variety of statistical methods that were applied to the sample of mussels. The statistical calculations were performed by the SPSS computer software.
- The selection of the statistical methods depended primarily on the types of the variables, since our data included different variables.
RESULTS

MUSSELS DATA AND MACROSCOPIC EXAMINATION

- 1280 adult Mediterranean mussels: 960 cultured and 320 wild were collected and included in the study.
- no mortality was detected in shellfish farms.
- the average length of cultured/wild mussels = 7/7.1 cm
- the average weight of cultured/wild mussels = 15/17.4 g
- the average condition index (CI) of cultured/wild mussels = 28.14/29.63
- only one severely emaciated mussel – 7.8 cm, 13.9 g, CI 14.39
HISTOPATHOLOGICAL EXAMINATION

- intracellular ciliates were detected in 293 mussels
- haemocytic neoplasia was detected in 14 mussels
- neither intracellular ciliates nor neoplasias nor inflammation were detected in the severely emaciated mussel
INTRACELLULAR CILIATES

- pear or spindle-shaped, 3.9 to 11.5 µm long and 2.9 to 8.4 µm width, with a polymorphic oval to globular basophilic, fragmented macronucleus,
- mostly found inside the digestive tubule epithelia, few were lying in the digestive tubules lumina,
• a slight enlargement of epithelial cells that harbored ciliates of a large size was observed, a mild diffuse haemocytic infiltration in digestive gland connective tissue was present in 6 (2%) and haemocytic neoplasia of mussels was noticed in 3 (1%) of infected mussels.
• the prevalence of infection was 22.9% (24.4% in cultured, 18.4% in wild – not significant),
• the prevalence was the highest in spring (42%) and the lowest in summer (12.7%) - significant.
Table: The intensity of infection with intracellular ciliates in cultured and wild mussels - not significant

<table>
<thead>
<tr>
<th>way of growing</th>
<th>mild</th>
<th>moderate</th>
<th>severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>cultured</td>
<td>76.5</td>
<td>14.5</td>
<td>9</td>
</tr>
<tr>
<td>wild</td>
<td>66</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>
Graph: the intensity of infection with intracellular ciliates in different months - significant
CONDITION INDEX (CI)

- CI: infected/healthy = 28.77/28.28 – not significant,

- ciliates were significantly more often found in longer and heavier mussels
Table: The condition index (CI) in infected and healthy cultured and wild mussels – not significant

<table>
<thead>
<tr>
<th>CI</th>
<th>health status</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>infected</td>
<td>healthy</td>
</tr>
<tr>
<td>way of growing</td>
<td></td>
<td>28.32</td>
<td>26.99</td>
</tr>
<tr>
<td>cultured</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wild</td>
<td></td>
<td>29.05</td>
<td>28.93</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>28.77</td>
<td>28.28</td>
</tr>
</tbody>
</table>
Table: The condition index of mussels according to different intensities of infection in cultured and wild mussels – not significant

<table>
<thead>
<tr>
<th>CI</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>cultured</td>
<td>28.85</td>
<td>28.61</td>
<td>28.09</td>
</tr>
<tr>
<td>wild</td>
<td>29.74</td>
<td>28.78</td>
<td>26.73</td>
</tr>
</tbody>
</table>
HAEMOCYTIC NEOPLASIA

- highly pleomorphic (spherical, oval, spindle and starry) and anisosocitotic (12.3-30.1 µm) neoplastic cells, with large, hyperchromatic and manly rounded but often also pleomorphic nucleus from 4.3-22.7 µm in diameter with finely dispersed or dense chromatin without nucleolus

haemocytic neoplasia in DG connective tissue, x 400
• some bi- or even tri-nucleated cells
• high nucleus to cytoplasm ratio; low number of mitosis (2 mitoses per HPF)
• diffuse infiltration of neoplastic cells in four, small foci in two and single neoplastic cells in 8 mussels

mitoses, single neoplastic cells, NH, x 400

diffuse form of NH, x 400

multifocal form of NH, x 200
necrosis and multifocal atrophy of digestive tubules in mussels with diffuse neoplasia and severe haemocytic infiltration of connective tissue in mussels with single neoplastic cells, no alterations in mussels with small foci of neoplastic cells.
• the prevalence of affection was 1.1% (1.25% in cultured, 0.6% in wild),
• the prevalence was the highest in spring and autumn (5 cases) and the lowest in winter (1 case).
Table: The number of haemocytic neoplasia (and the distribution of neoplastic cells: S, M, D) throughout the year in cultured and wild mussels

<table>
<thead>
<tr>
<th>month</th>
<th>cultured (n)</th>
<th>wild (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>February</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March</td>
<td>2 – S + D</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>3 – S(2) + D</td>
<td>0</td>
</tr>
<tr>
<td>June</td>
<td>2 - S</td>
<td>0</td>
</tr>
<tr>
<td>July</td>
<td>1 - M</td>
<td>0</td>
</tr>
<tr>
<td>August</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>September</td>
<td>1 - M</td>
<td>2 - S</td>
</tr>
<tr>
<td>October</td>
<td>2 – D+S</td>
<td>0</td>
</tr>
<tr>
<td>November</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>December</td>
<td>1 - D</td>
<td>*</td>
</tr>
</tbody>
</table>
Table: The condition index (CI) in affected and healthy cultured and wild mussels

<table>
<thead>
<tr>
<th>CI</th>
<th>health status</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>way of growing</td>
<td>affected</td>
<td>healthy</td>
</tr>
<tr>
<td>cultured</td>
<td></td>
<td>30.68</td>
<td>28.1</td>
</tr>
<tr>
<td>wild</td>
<td></td>
<td>26.95</td>
<td>28.95</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>30.1</td>
<td>28.3</td>
</tr>
</tbody>
</table>
Table: CI in different distribution of neoplastic cells

<table>
<thead>
<tr>
<th>CI - the distribution of neoplastic cells</th>
<th>single</th>
<th>focal</th>
<th>diffuse</th>
</tr>
</thead>
<tbody>
<tr>
<td>single</td>
<td>30.26</td>
<td>33.05</td>
<td>28.3</td>
</tr>
</tbody>
</table>

Graph: prevalence of intracellular ciliates and haemocytic neoplasia in different months in correlation with sea temperature, salinity and oxygenation
CONCLUSIONS

- Slovene Mediterranean mussels (*Mytilus galloprovincialis*) are infected with intracellular ciliates of mussels and affected with haemocytic neoplasia of mussels.
- The prevalence of infection with intracellular ciliates of mussels in Slovene Mediterranean mussels is moderate, the intensity of the infection is predominantly mild.
- The infection with intracellular ciliates of mussels causes only a slight enlargement of epithelial cells that carried ciliates of a large size.
- The infection with intracellular ciliates of mussels is not seasonal and is the highest in spring and the lowest in summer.
• The infection with intracellular ciliates is not linked to the way of mussels grooving, neither to the months of the year.
• The salinity has negative influence on the infection with intracellular ciliates, whereas sea temperatures and oxygenation have no influence on the infection.
• Intracellular ciliates more often inhabit heavier and longer mussels, but have no influence on the condition index of infected mussels.
- Haemocytic neoplasia of mussels occurs only sporadically in Slovene Mediterranean mussels.
- Diffuse haemocytic neoplasia causes necrosis and multifocal atrophy of digestive tubules, single and multifocal neoplastic cells have no influence on the digestive gland.
- The affection is not seasonal and is the highest in spring and in autumn and the lowest in winter.
- The number of mussels with haemocytic neoplasia of mussels was too small to enable reliable statistical analysis.