

OCCURRENCE AND EFFECTS OF THE BIVALVE-INHABITING HYDROID *EUGYMNANTHEA INQUILINA* IN CULTURED MEDITERRANEAN MUSSELS (*MYTILUS GALLOPROVINCIALIS*) IN SLOVENIA

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Summary: The Mediterranean mussel (*Mytilus galloprovincialis*) is the most common bivalve species bred in the Slovenian sea. The mussels are bred on three locations, i.e., Seča, Strunjan and Debeli rtič in natural fisheries–shellfish farms. From November 2007 to October 2008, 960 adult cultured Mediterranean mussels were randomly sampled and included in our study. Water temperature, oxygenation and salinity were measured at each sampling. The mussels were measured and weighted to calculate the condition index and microscopically examined for the presence of hydroids. No increased mortality occurred during the sampling. Hydroids of *Eugymnanthea inquilina* were detected with 60.6% prevalence, mostly during summer. They were either attached to the mantle epithelium or found inside the mantle cavity. No alterations were observed at the point of attachment. The differences in the prevalence of infection in different seasons were statistically significant. Oxygen content and salinity had a statistically significant impact on the presence of infection, while temperature did not. The average condition index of mussels inhabited by *E. inquilina* was significantly lower than in uninfected mussels, indicating a parasitic relationship between *Mytilus galloprovincialis* and *Eugymnanthea inquilina*.

This is the first report on the occurrence of *E. inquilina* in Mediterranean mussels from the Slovenian sea.

Key words: *Eugymnanthea inquilina*, *Mytilus galloprovincialis*, histopathology, condition index, Gulf of Trieste

Introduction

The Slovenian sea, part of the Gulf of Trieste, the northernmost end of The Adriatic Sea, represents a specific and rich habitat, highly influenced by considerable fluctuations of temperature, oxygenation and salinity. The Mediterranean mussel (*Mytilus galloprovincialis*) is one of the most common bivalve species in the Slovenian sea and the most often bred bivalve. The mussels are bred on three locations, i.e., Seča, Strunjan and Debeli rtič in natural fisheries–shellfish farms.

The farms are quite small and composed of 5 fields, each measuring 20 000 m². The annual production of each field is from 25 to 30 tons. The high density of mussels in aquacultures can lead to the spread of several epizootic diseases, which can affect the revenues and farming production. One of the endobionts found in Mediterranean mussels is the hydroid *Eugymnanthea inquilina* (Cnidaria, Hydrozoa). It has so far been reported in Greece (1, 2), Italy (3), Croatia (4) and Japan (5), but no record of this species exists in Slovenia. The athenate solitary hydroid stage of *E. inquilina* attaches itself with a basal disc in the mantle cavity of *M. galloprovincialis* and other bivalves (3). It is unclear what, if any, are the effects of this organisms on the survival and condition of the host

organism. Kubota (6) hypothesises that the relationship between the host and endobiont is commensalism with no harmful or beneficial effects to either organism. On the other hand, Rayyan et al. (2) and Galinou-Mitsoudi et al. (7) conclude that a parasitic relationship is more likely, since the presence of hydroids was correlated with lower growth rates and condition indices of the mussels. On the other hand, Mladineo et al. (8) found no deteriorating impact on host reproduction or condition index, but did observe ultrastructural alterations (cell desquamation) in host cells at the attachment site. The possibility of mutualism between *E. inquilina* and *M. galloprovincialis* has also been proposed; Piraino et al. (3) found an indication that the hydroids may have a protective role against mussel parasites.

In order to confirm the presence of *Eugymnanthea inquilina* in Mediterranean mussels from the Slovenian sea, we collected and histologically examined mussels from two shellfish farms. We measured the mussel condition index in order to ascertain potential effects of the hydroid on the condition of the farmed mussels. To observe any effects of environmental conditions on the presence and abundance of the *E. inquilina* polyps, we also measured water temperature, salinity and oxygenation. The prevalence of *E. inquilina* was correlated to the presence of intracellular ciliates in the same samples (9) in order to ascertain whether the presence of *E. inquilina* has any effect on the prevalence of other endobionts in the Mediterranean mussel. This is the first report on the occurrence of this organism in Slovenia.

Materials and methods

80 adult cultured Mediterranean mussels (*Mytilus galloprovincialis*) from shellfish farms in Seča and Strunjan were stripped directly from ropes at a depth of approximately 3 metres monthly from November 2007 to October 2008. In total, 960 adult Mediterranean mussels were collected and included in our study. Water temperature, oxygenation and salinity were measured at each sampling at the depth of sampling (3 m). Oxygenation measurements were not performed in March. Water temperature and oxygenation were measured using a thermometer "MultiLine P4 – Oxi 320 Set" with a dissolved oxygen probe "Cellox 325" (WTW). Water salinity was measured using a hand-held

refract meter "S/Mill-E. S= 0-100‰" (ATAGO).

Live adult mussels were transported to the laboratory within one hour in a classic cooling bag. Organisms attached to the shell were carefully removed and the shells were washed with fresh water.

The length of the mussels was measured from the hinge to the longest part of the shell. The shell was opened and excess water was removed. The total weight of each mussel was measured and the flesh was carefully removed, drained on double absorbent paper and weighed. The total weight and weight of the flesh were determined to ± 0.01 g with an electronic balance. The flesh condition index was calculated by means of the formula "condition index = fresh flesh weight \times 100/total weight".

A standard section through the visceral mass was performed after weighing. Samples were immediately placed in 10% formalin solution at room temperature for no more than 24 hours and were routinely paraffin embedded. Four- μ m-thick sections were stained with haematoxylin and eosin (HE) and one slide per mussel was examined under a light microscope for the presence of hydroids.

Morphometric analyses were performed on tissue slide photographs using a digital camera and Microphot microscope. Measurements of hydroids were performed using the computer programme NIS-Elements BR. The length of fifty randomly chosen hydroids was measured and the average value of the measured parameters was calculated.

The statistical calculations were performed using the SPSS software package. The independent samples t-test was used to compare the condition indices of infected and uninfected mussels. To analyse the possible independent effects of temperature, oxygenation and salinity on the occurrence of infection, we used the binary logistic regression. The chi-square test of independence was employed to examine the occurrence of simultaneous infections with *E. inquilina* and intracellular ciliates.

Results

1. Occurrence of *Eugymnanthea inquilina* in Slovenian Mediterranean mussels

No increased mortality was detected in shellfish farms during the one year sampling period.

The length of the mussels varied from 5.0 to 9.8 cm (average 7.0 cm).

The total weight of mussels varied from 5.0 to 39.5 g (average 15 g) whereas the weight of the flesh varied from 1.2 to 12.2 g (average 4.15 g). The condition index varied from 11.17 to 69.33 (average 28.14).

Overall, the prevalence of hydroid infection was 60.6%. The hydroids (Figure 1) were 111–664 μm long (average 345 μm). They were either attached to the mantle epithelium or found inside the mantle cavity (Figure 1). No alterations were observed at the point of attachment.

The average condition index of infected mussel was 28.3 and it was lower than that of healthy ones (29.8). The difference in the condition index between infected and healthy mussels was statistically significant ($t = 4.095$; $p < 0.001$).

2. Correlation with environmental parameters

The measured sea temperatures, oxygenation and salinity in relation to hydroid prevalence are presented in figure 2.

The highest average prevalence of infection (82.7%) was detected in summer (June, July, August) with an average sea temperature of 24.1 $^{\circ}\text{C}$, oxygenation of 7.6 mg/l and salinity of 38.1‰. The lowest prevalence (37.0%) was observed in winter (December, January, February) with an average

sea temperature of 9.1 $^{\circ}\text{C}$, average oxygenation of 11.6 mg/l and average salinity of 37.25‰. The highest absolute prevalence was detected in September (86.7%) with a sea temperature of 20.3 $^{\circ}\text{C}$, oxygenation of 6.9 mg/l and salinity of 39‰, and the lowest in March (20%) with the sea temperature of 11.1 $^{\circ}\text{C}$ and salinity of 32.5‰.

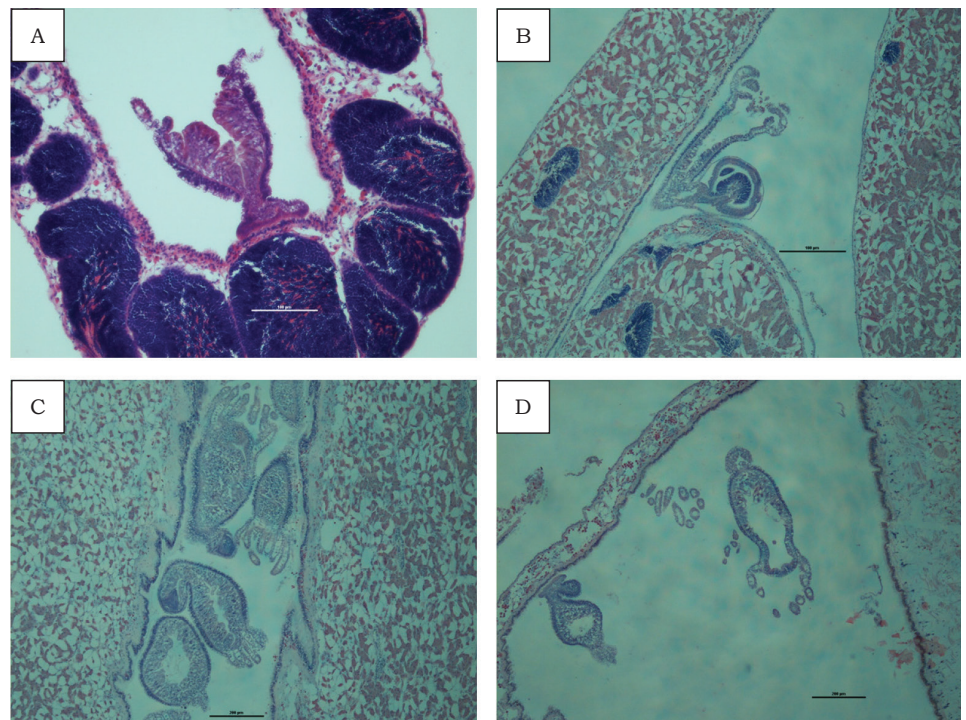
The differences in the infection prevalence between the months of sampling were statistically significant ($V = 0.445$; $p < 0.001$). The binary logistic regression showed that oxygen content and salinity have a statistically significant ($p < 0.001$) correlation with the presence of infection, while temperature does not (Table 1).

3. Concurrent infection with intracellular ciliates of mussels

The occurrence of intracellular ciliates of mussels and *E. inquilina* in all the sampled mussels is summarised in Table 2.

A chi-square test of independence was performed to examine the relation between the occurrence of intracellular ciliates in mussels with or without hydroids. The relation between the variables was significant, $\chi^2 (1, N = 960) = 5.96$, $p = 0.015$. Intracellular ciliates are less likely to invade mussels with *Eugymnanthea inquilina*.

Figure 1: *Eugymnanthea inquilina* in the mantle cavity of Mediterranean mussels. A: *E. inquilina* polyp attached to the mantle, HE staining, $\times 100$; B: *E. inquilina* polyp with a medusoid bud surrounded by a membrane in the mantle cavity, HE staining, $\times 40$; C: Numerous *E. inquilina* polyps in the mantle cavity, HE staining, $\times 40$; D: Two *E. inquilina* polyps, one with a medusoid bud attached to the mantle, another in the mantle cavity, HE staining, $\times 40$



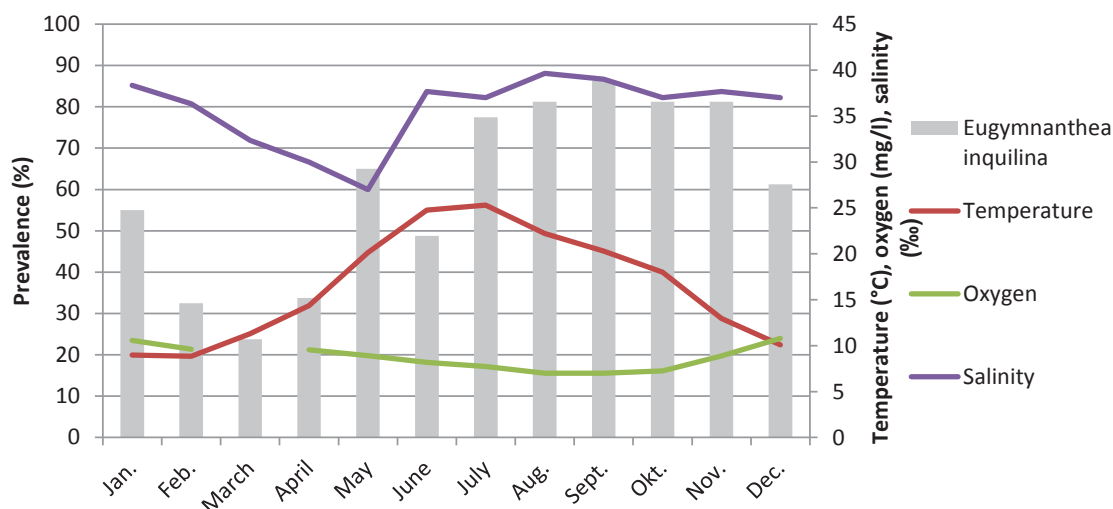


Figure 2: Prevalence of hydroids in different months in correlation with sea temperature, salinity and oxygenation

Table 1: Results of the binary logistic regression analysing the possible independent effects of temperature, oxygenation and salinity on the occurrence of infection.

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1(a)	T ^a	,001	,018	,003	1	,960	1,001
	O ^b	-,335	,082	16,512	1	,000	,715
	S ^c	,060	,017	12,481	1	,000	1,062
	Constant	1,329	1,331	,997	1	,318	3,777

^a Temperature ^bOxygen concentration; ^cSalinity

Table 2: The observed numbers of Mediterranean mussels infected with *Eugymnanthea inquilina* and intracellular ciliates of mussels

Intracellular ciliates	<i>Eugymnanthea inquilina</i>		Total
	Yes	No	
Yes	126	108	234
No	456	270	726
Total	582	378	960

Discussion

Our research is the first to demonstrate that *Eugymnanthea inquilina* is present in cultured Mediterranean mussels from the Slovenian sea. The average incidence of infection with the hydroid was 60.6%. This is comparable to the prevalence of 73.9% reported in the Ionian Sea, Italy (3) and 48.3% (2) and up to 78.1% (7) in the North Aegean Sea, Greece.

We observed statistically significant differences in the prevalence of infection with *Eugymnanthea*

inquilina in different seasons. The highest prevalence was observed during summer and autumn months, which is contradictory to the data reported in Rayyan et al. (2) and Piraino et al. (3), who, for the same part of the year, both found a decrease in prevalence. Oxygen content and salinity were shown to have a statistically significant ($p < 0.001$) correlation with the presence of infection with *E. inquilina*, while temperature did not. Increased salinity and decreased oxygen are probably stressful conditions for the host, which could explain the increased prevalence of the medusoid.

Since no increased mortality occurred in shellfish farms during the year of sampling, we can conclude that the effects of *Eugymnanthea inquilina* on the host organism are not lethal. The condition index of invaded mussels was significantly lower than that of healthy ones. This implies that serious invasions of *Eugymnanthea inquilina* in mussel farms could mean a decrease in the production and revenues of the farms.

The mussels infected with *Eugymnanthea inquilina* were less likely to contain intracellular ciliates than hydroid-free mussels. Our previous research (9) has shown that the difference in the condition indices between mussels infected with ciliates and healthy ones was not statistically significant. However, ciliates were most often observed in longer and heavier mussels. We concluded that intracellular ciliates more frequently inhabit older mussels in better condition and are harmless commensals when present in small numbers, but a more numerous infection causes a decrease in the mussel's condition index. The observed decrease in the occurrence of intracellular ciliates in mussels infected with *Eugymnanthea inquilina* could therefore be an indirect consequence of decreased condition index of the mussels. It is also possible that *Eugymnanthea inquilina* has some direct effect on the presence of intracellular ciliates of mussels (e.g. by excreting repellent substances), but this possibility needs to be researched further.

On the basis of our research we can conclude that *Eugymnanthea inquilina* is present in Slovenian shellfish farms to a relatively large extent. The interaction between the host and endobiont is not mere commensalism since infection with *E. inquilina* decreases the condition of affected mussels. Similar to some of the other authors (2, 7), we can conclude that the relationship between Mediterranean mussels and hydroids is more likely to be parasitism. An increase in the prevalence of *Eugymnanthea inquilina* in shellfish farms could therefore potentially represent a threat to their production. It is recommended that the condition of the infection be monitored in the future in order to have a comprehensive warning system for potential decline in the condition and growth of *Mytilus galloprovincialis* in the Slovenian sea.

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References

1. Rayyan A, Christidis J, Chintiroglou CC. First record of the bivalve-inhabiting hydroid *Eugymnanthea inquilina* in the eastern Mediterranean sea (Gulf of Thessaloniki, north Aegean sea, Greece). J Marine Biol Assoc United Kingdom 2002; 82(5): 851-3.
2. Rayyan A, Photis G, Chintiroglou CC. Metazoan parasite species in cultured mussel *Mytilus galloprovincialis* in the Thermaikos gulf (North Aegean sea, Greece). Dis Aquat Organ 2004; 58(1): 55-62.
3. Piraino S, Todaro C, Geraci S, Boero F. Ecology of the bivalve-inhabiting hydroid *Eugymnanthea inquilina* in the coastal sounds of Taranto (Ionian sea, Se Italy). Marine Biol 1994; 118(4): 695-703.
4. Mladineo I, Petrić M, Bočina I. Harmful epibiosis of *Eugymnanthea inquilina* (Cnidaria, Hydrozoa) and doubtful parasitism of *Urastoma cyprine* (Turbellaria, Holocoela) in the *Mytilus galloprovincialis* (Bivalva). In: Diseases of fish and shellfish. 15. EAFP Conference of fish and shellfish. Split : Dalmacijapapir, 2011: 287.
5. Kubota S. Occurrence of a commensal hydroid *Eugymnanthea inquilina* Palombi from Japan. J Fac Sci Hokkaido Univ 1979; 21(4): 396-406.
6. Kubota S. Studies on life history and systematics of the Japanese commensal hydroids living in bivalves, with some reference to their evolution. J Fac Sci Hokkaido Univ 1983; 23(3): 296-402.
7. Galinou-Mitsoudi S, Giannakourou A, Petridis D, Papathanasiou E. Mussel endobionts in Greek cultures: first occurrence and effects. In: 1st International Symposium of Aquacultures, Fisheries Technology & Environmental Administration. Athens, 2002: 1-11.
8. Mladineo I, Petric M, Hrabar J, Bocina I, Peharda M. Reaction of the mussel *Mytilus galloprovincialis* (Bivalvia) to *Eugymnanthea inquilina* (Cnidaria) and *Urastoma cyprinae* (Turbellaria) concurrent infestation. J Invertebr Pathol 2012; 110(1): 118-25.
9. Gombač M, Makarovič M, Fonda I, Jenčič V. The influence of sea temperature, salinity and oxygenation on intracellular ciliates dynamics in Slovene Mediterranean mussels (*Mytilus galloprovincialis*). Bull Eur Assoc Fish Pathol 2011; 31(2): 66-72.

POJAVLJANJE IN UČINKI TRDOŽIVNJAKA *EUGYMNANTHEA INQUILINA* V GOJENIH MEDITERANSKIH KLAPAVICAH (*MYTILUS GALLOPROVINCIALIS*) V SLOVENIJI

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Povzetek: Mediteranska klapavica (*Mytilus galloprovincialis*) je v slovenskem morju najštevilčnejša in najpogostejše gojena vrsta školjk. Gojijo jo na treh lokacijah, pri Seči, Strunjanu in Debelem Rtiču. V našo raziskavo smo vključili 960 klapavic, ki smo jih naključno vzorčili v obdobju med novembrom 2007 in oktobrom 2008 v školjčiščih pri Seči in Strunjanu. Med vsakim vzorčenjem smo izmerili temperaturo morja, koncentracijo raztopljenega kisika in slanost vode. Školjke smo izmerili in stehtali, jim izračunali kondicijski indeks in jih s histopatološko preiskavo pregledali na prisotnost trdoživnjakov. Med vzorčenjem v školjčiščih nismo opazili poginov školjk. Trdoživnjaka *Eugymnanthea inquilina* smo diagnosticirali v 60,6 % školjk, največjo prevalenco smo zaznali poleti. Trdoživnjaki so bili pritrjeni na plašč ali pa smo jih našli proste v plaščevi votlini. Na mestu pritrditve nismo opazili nobenih tkivnih sprememb. Razlike v prevalencah v različnih letnih časih so bile statistično značilne. Koncentracija raztopljenega kisika in slanost sta značilno vplivali na prisotnost trdoživnjakov, temperatura pa ne. Povprečen kondicijski indeks klapavic s trdoživnjaki je bil statistično značilno nižji od kondicijskega indeksa zdravih klapavic, kar kaže na možnost parazitizma trdoživnjaka *Eugymnanthea inquilina*.

To je prvo poročilo o prisotnosti endobiontskega trdoživnjaka *E. inquilina* v mediteranskih klapavicah v slovenskem morju.

Ključne besede: *Eugymnanthea inquilina*; *Mytilus galloprovincialis*; histopatologija; kondicijski indeks; Tržaški zaliv