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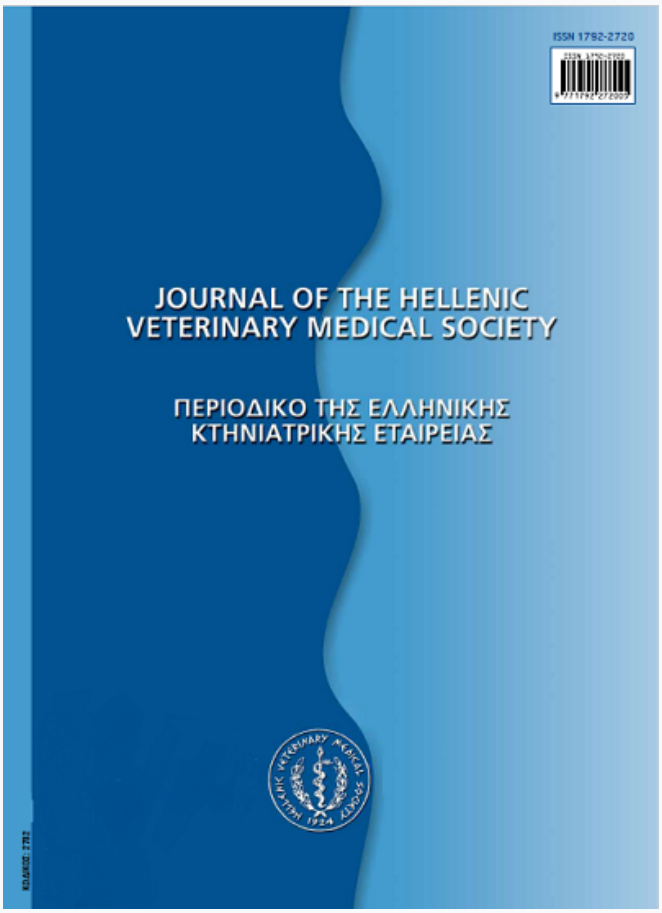

Effect of culture system on the prevalence of parasites of the Mediterranean mussel Mytilus galloprovincialis (Lamark, 1819)

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Effect of culture system on the prevalence of parasites of the Mediterranean mussel *Mytilus galloprovincialis* (Lamark, 1819)

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Επίδραση του συστήματος καλλιέργειας στο ποσοστό προσβολής του Μεσογειακού μυδιού *Mytilus galloprovincialis* (Lamark, 1819) από παράσιτα

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ABSTRACT. For the production of the Mediterranean mussel, *Mytilus galloprovincialis*, two systems are used in Greece, the 'long-line' and the 'on-table' system. In the present study, effects of farming system on prevalence of infection by some parasites of Mediterranean mussels were investigated. Three mussel culture sites, located at Thermaikos gulf, with similar profiles, where both farming systems are practiced, were selected. Two samplings took place in 2008, one in July and one in October. From each site, 30 mussels per culture system were collected. From each mussel, tissue samples from the visceral mass, mantle and gills were collected and examined histologically for the presence of any parasites. Number of mussels parasitized by at least one parasitic species was substantially increased in mussels cultured with the 'on-table' system. In particular, prevalence of infection by *Urastoma cyprinae* and *Marteilia* spp. was substantially increased in mussels cultured with the 'on-table' system, while no relation between farming system and prevalence of infection by *Eugymnanthea inquilina* was observed. Prevalence of infection by *Myticola intestinalis*, *Steinhausia mytilovum* and *Proctoeces maculatus* was considerably low in both culture systems. In addition, in both culture systems, number of the mussels infected by E. *inquilina* and *U. cyprinae* was substantially increased in October, while an opposite trend was noted for *Marteilia* spp.

Keywords: culture system, *Mytilus galloprovincialis*, parasite

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ΠΕΡΙΛΗΨΗ. Στην παραγωγή του Μεσογειακού μυδιού *Mytilus galloprovincialis*, τα δύο συστήματα καλλιέργειας που εφαρμόζονται στην Ελλάδα, είναι οι πλωτές εγκαταστάσεις ('long-lines') και οι πασσαλωτές εγκαταστάσεις ('on-tables'). Στην παρούσα μελέτη, εξετάστηκε η επίδραση του συστήματος καλλιέργειας στο ποσοστό των μυδιών που παρασιτούνται από διάφορα παράσιτα. Για τον σκοπό αυτό, επιλέχτηκαν τρεις περιοχές στο Θερμαϊκό κόλπο, με παρόμοια χαρακτηριστικά και στις οποίες εφαρμόζονται και τα δύο συστήματα καλλιέργειας. Πραγματοποιήθηκαν δύο δειγματοληψίες, τον Ιούλιο και τον Οκτώβριο 2008. Από κάθε περιοχή, συλλέχθηκαν 30 μύδια ανά σύστημα καλλιέργειας και από κάθε μύδι λήφθηκαν δείγματα ιστών για ιστολογική εξέταση από το σπλαχνικό σάκο, τα βράγχια και το μανδύα, προκείμενου να διαπιστωθεί η παρουσία παρασίτων. Ο αριθμός των μυδιών που παρασιτούνταν από τουλάχιστον ένα είδος παρασίτου ήταν σημαντικά αυξημένος σε μύδια που καλλιεργούνταν με το πασσαλωτό σύστημα. Πιο συγκεκριμένα, το ποσοστό των μυδιών που παρασιτούνταν από τα παράσιτα *Urastoma cyprinae* και *Marteilia* spp. ήταν σημαντικά αυξημένο στα μύδια που καλλιεργούνταν με το πασσαλωτό σύστημα, ενώ δεν υπήρχε σχέση μεταξύ του συστήματος καλλιέργειας και του ποσοστού παρασίτωσης από *Eugymnanthea inquilina*. Το ποσοστό παρασίτωσης από τα παράσιτα *Myticola intestinalis, Steinhaus mytilovum* και *Proctoeces maculatus* ήταν πολύ χαμηλό και στα δύο συστήματα καλλιέργειας. Επιπλέον, και στα δύο συστήματα καλλιέργειας, το ποσοστό των μυδιών που παρασιτούνταν από τα παράσιτα *E. inquilina και U. cyprinae* αυξήθηκε σημαντικά τον Οκτώβριο, ενώ η αντίθετη τάση παρατηρήθηκε για το παράσιτο *Marteilia* spp.

Λέξεις ευρετηρίασης: μέθοδος καλλιέργειας, παράσιτα, *Mytilus galloprovincialis*

INTRODUCTION

Culture of the Mediterranean mussel *Mytilus galloprovincialis* in Thermaikos gulf (northern Greece) has been an important activity since 1980. In that area, over 200 mussel farming sites exist, producing close to 30,000 tonnes of mussels (Theodorou et al., 2011). Most farms (about 130) use the 'long-line' culture system. Sea depth at those sites ranges between 8 and 20 m. In sea depths between 3-4 m, the 'on-table' system is applied (about 85 sites). In both systems, mussels grow within cylindrical nets attached to either horizontal ropes ('long-line') or horizontal poles or tubes ('on-table'). Main difference between the two systems is the sea depth at which culture takes place and the fact that in the 'on-table' system, mussels can be exposed to the air for long periods of time to control fouling (Angelidis, 2007); exposure to air and sun can kill many of the symbionts that are attached on the valves of the mussels and could inhibit their growth. In terms of production, in 'long-line' sites production amounts to 100-150 tonnes per hectare, while in 'on-table' sites production amounts up to 500 tonnes per hectare (Angelidis, 2007).

A number of parasites can infect Mediterranean mussels, with *Marteilia refrigens* being the most important one, as infection by that organism is listed in the OIE**'s** list of notifiable diseases (Francisco et al., 2010). Until now however, most studies related to parasites of the Mediterranean mussel have focused on seasonal fluctuations of the parasitism or on association of various geographical areas and sea depths with presence of parasitic species (Robledo and Figueras, 1994; Robledo et al., 1994; Rayyan et al., 2004; Francisco et al., 2010). With the exception of one published study (Karagiannis and Angelidis, 2007) on *Marteilia* spp., there are no published reports on the overall effect of the system used for culture of the Mediterranean mussel on prevalence of individual parasitic species and on total parasitic load. In the study by Karagiannis and Angelidis (2007), mussels cultured with the 'on-table' system, had increased prevalence of the infection by *Marteilia* spp. However, the study did not provide any information on prevalence of other parasites. In other mussel species, as for example in blue mussel, *Mytilus edulis*, previous studies have already indicated a positive relation between inshore culture system and number of parasitized mussels, as well as intensity of parasitism (Buck et al., 2005).

Objective of the present study was to assess potential association of prevalence of the most common parasites of Mediterranean mussels with the two culture systems ('long-line' and 'on-table') used in Greece was investigated. Three sites located at Thermaikos gulf, where both systems are used, were selected and mussels from both systems were sampled and examined for presence of parasites. Two samplings took place, one in the summer (July) and one in the autumn (October); in each sampling, total number

of parasitized mussels and prevalence of the various parasitic species identified were recorded. Previous studies (Rayyan and Chintiroglou, 2003; Rayyan et al., 2004; Karagiannis and Angelidis 2007) have indicated that most parasites of cultured Mediterranean mussels in the Thermaikos gulf occur throughout the year and, especially during the months when sampling took place, their prevalence had significant differences, probably due to their life cycles. Thus, these two periods were selected in order to examine possible effects t of the culture system on the prevalence of the parasites during the periods of low and high prevalence.

MATERIALS AND METHODS

Site selection and samplings

Thermaikos gulf is a closed nutrient-rich basin in the northwest section of the Aegean Sea. In the present study, three mussel culture sites, were selected (Fig. 1). These sites were located close to the western coastline of the Thermaikos gulf and in these sites both culture systems were used. The area of location of the three culture sites was close to the deltaic system of Axios river, which supplies over 50% of total fresh water of the gulf. The distance between the two culture systems was approximately 0.5 nautical miles. The water in that area is particularly rich in organic materials and exhibits significant annual and daily variations in some water quality parameters. According to Krestenitis et al. (2012), annual water temperature ranges from 7 ºC (winter) to over 28 ºC (summer), while salinity ranges between 34 to 38 ppt. Water depth at the sites of the 'on-table' system was 3-4 m, while that of the 'long-line' system was about 15 m.

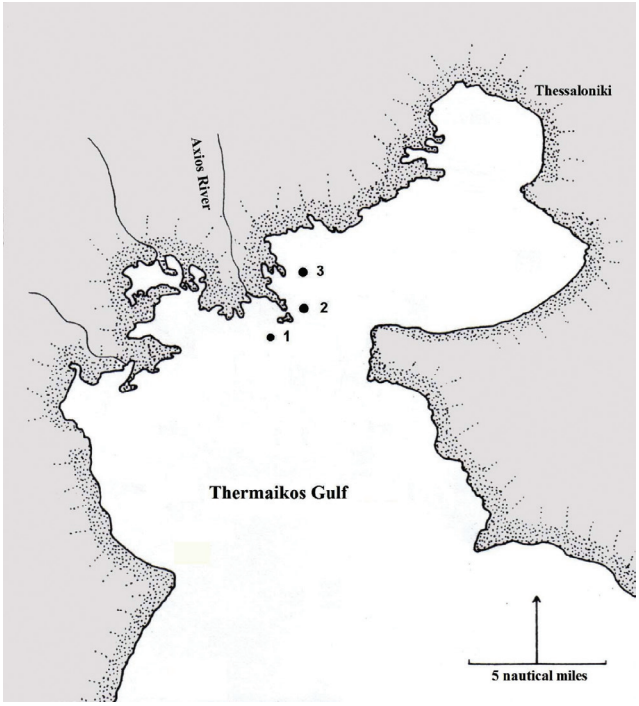

Fig. 1. Map of Thermaikos gulf, Northern Greece, with the farming areas that were included in the present study.

Two samplings took place at each culture site. First sampling was performed in July 2008 and the second in October of the same year. Samplings took place between 10:00 and 12:00; in each sampling, 30 live mussels from each culture system (i.e., 60 mussels per sampling site), with a shell length of 50-54 mm, were collected. In all cases and at each culture system, 5 mussels were randomly collected from the middle section of 6 different hanging nets (mean length 2.5 m). All mussels were transported for detailed examination in ice package. During sampling, temperature, salinity, oxygen concentration and pH of the water at each sampling site were recorded.

Mussels were cleaned externally and their valves were separated using a knife. Tissue samples from the visceral mass, mantle and gills were removed and placed into Davidson's fixative (Shaw and Battle, 1957), with a ratio of 1 volume of tissue to 10 volumes of fixative, for 48 h. Samples were processed using the histological procedures described by Bancroft and Gamble (2007) and finally embedded in paraffin wax. Two non-consecutive thin sections (5 μm) were cut for each tissue sample, stained with haematoxylin-eosin and examined using light microscopy. Parasites were identified on the basis of their morphology.

Statistical analysis

Prevalence of parasites was measured as the percentage of infected mussels within the selected sample. Association of parasitic infection to production system, time and site was evaluated using the chi-square test of independence (χ^2-test). The chi-square test of independence was applied to determine whether there was a significant association between two variables or not. The same test was applied in order to examine a relation between prevalence of each parasitic species and production system used for culture of mussels. Statistical analysis was performed by using the SPSS software program; significance was set at $P \leq 0.05$, unless otherwise noted.

RESULTS

Water quality parameters

Mean (± standard deviation) values of water quality parametres were as follows: water temperature 25±0.2 ºC and 18±0.1 ºC, salinity 35±0.2 ppt and 34±0.3 ppt, oxygen concentration 6.2±0.4 ppm and 7.3±0.3 ppm, pH 7.8±0.1 and 7.9±0.2, for the July and October sampling, respectively.

Localisation and identification of parasites found in the Mediterranean mussels

Four metazoan parasites (*Eugymnanthea inquilina*, *Urastoma cyprinae*, *Mytilicola intestinalis* and *Proctoeces maculatus*) and two protistan parasites (*Steinhausia mytilovum* and *Marteilia* spp.) were identified in mussels collected during the study. Among these, *E. inquilina*, *Marteilia* spp. and *U. cyprinae* were those more frequently recorded.

Polyps of *E. inquilina* were seen within the mantle cavity of the mussels, swimming freely in the intravalve water or attached with their pedal disks to the mantle, the labial palps and the foot (Fig. 2a). Polyps were 0.7-3 mm long; some of them had developed medusa bud. No host injury was noted.

The turbellarian *U. cyprinae* was found among filaments of the demibranches, inside the mantle cavity (Fig. 2b). Shape of this gill worm varied between round and oval; its length was 0.3 to 0.8 mm. Haemocytic infiltration of gill tissue was found surrounding the area where the parasite was attached.

The copepod *M. intestinalis* was detected in the gut and in the stomach (Fig. 2c) of mussels. Mild haemocytic lesions were observed in the connective tissue surrounding the site of the infection.

Sporocysts containing developing cercariae of the digenean trematode *P. maculatus* were mainly found in the mantle, the digestive glands, the gills and the foot of the infected mussels (Fig. 2d). Strong haemolysis in the affected tissues was observed, which used to encapsulate and destroy the sporocysts.

The microsporidian *S. mytilovum* affected only female mussels. Sporocysts were detected in the cytoplasm of some mature ovum and not in their nucleus (Fig. 2e) and appeared spherically shaped, 10-15 pm in diametre, containing 15-30 spherical spores. Presence of *S. mytilovum* in ova of mussels was accompanied by strong haemolysis in the connective tissue surrounding the affected gonadal follicles.

The paramyxean parasite *Marteilia* spp. was seen in epithelial cells of the stomach (plasmodia) and the digestive tubules, where sporulation occurred (Fig. 2f). Epithelia of the digestive glands appeared to be destroyed, due to release of sporangia in the lumen of the digestive tubules. Haemolysis in the connective tissue of the digestive glands was also observed.

Effect of culture system and season on the prevalence of the parasites found in the Mediterranean mussels

Table 1 presents the prevalence of each parasite identified in the samples in terms of production system and sampling period. The results of the statistical analysis regarding influence of the culture site on presence of parasites in mussels indicated that there was no statistically significant effect and hence, no individual values for each site are presented. It should be mentioned though, that the rate of infected mussels did not vary substantially between sites neither in total, nor in each production system.

Overall number of mussels parasitized by at least one parasitic species was substantially increased in the mussels cultured with the ‘on-table’ system compared to those cultured with the ‘long-line’ system ($\chi^2 = 25.027$, $P < 0.001$). Number of parasitized mussels was considerably increased in the second sampling in both culture systems.

The results of evaluation of the relation between prevalence of each parasitic species and production system used for culture of mussels indicated that the production system had a statistically significant effect on the presence of *U. cyprinae* and *Marteilia* spp. ($\chi^2 = 13.046$, $P < 0.001$ and $\chi^2 = 14.755$, $P < 0.001$, respectively) in the mussels. Prevalence of *U. cyprinae* and *Marteilia* spp. was higher in mussels cultured with the ‘on-table’ production system. On the other hand, no statistically significant relation was observed between culture system and prevalence of *E. inquilina* ($\chi^2 = 0.012$, $P = 0.913$). Proportion of mussels infected with parasite was 36% (65 of 180 mussels) and 37% (66 of 180 mussels) in the ‘long-line’ and the ‘on-table’ system, respectively. Number of mussels parasitized by *M. intestinalis*, *S. mytilovum* and *P. maculatus* was considerably low in both culture systems, hence, no statistical analysis was applied for those species.

Differences in number of parasitized mussels were evident between the two samplings (Table 1). In both culture systems, the number of the mussels infected by E. *inquilina* and *U. cyprinae* increased substantial-

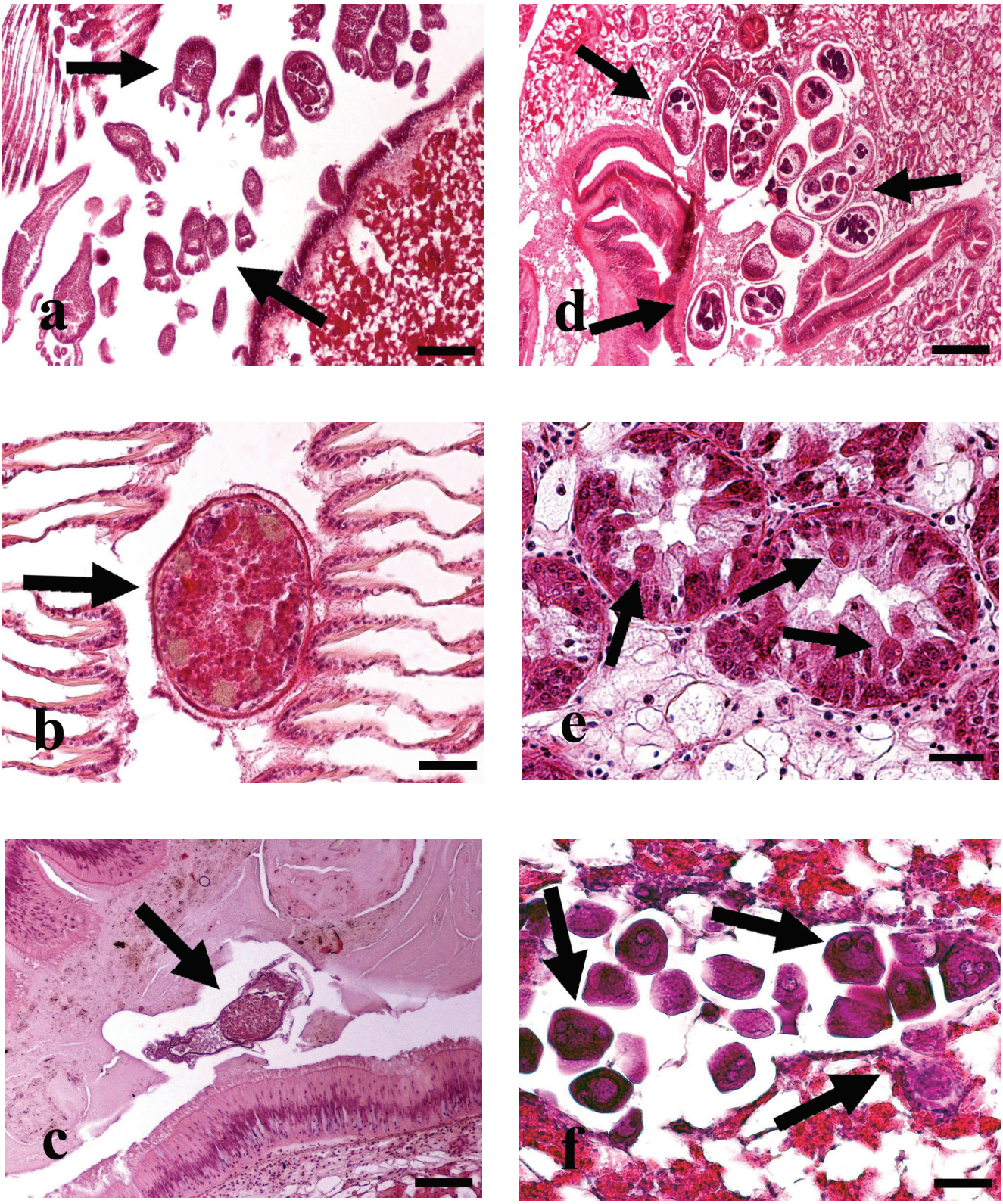

Fig. 2. Various parasitic species identified in *Mytilus galloprovincialis* (haematoxylin-eosin stain). (a) Polyps of *Eugymnanthea inquilina* in the mantle cavity (bar: 250 μm); (b) *Urastoma cyprinae* among the brachial filaments (bar: 50 μm); (c) *Marteilia intestinalis* in the lumen of stomach (bar: 50 μm); (d) Sporocysts of *Proctoeces maculatus* in digestive glands (bar: 50 μm); (e) M*arteilia* spp. in the epithelium of digestive tubules (bar: 25 μm); (f) Cysts of *Steinhausia mytilovum* in the ovum cytoplasm (bar: 50 μm).

Table 1. Prevalence of infection by parasitic species identified in Mediterranean mussels, collected on two sampling occasions from three different sites of the Thermaikos gulf; in each site, farming sites applying one of two different production systems were included in the study.

Site	Total	Production system					
		'Long-line' system			*'On-table' system*		
		July sampling	October sampling	Total	July sampling	October sampling	Total
Number of mussels parasitized by at least one parasitic species							
1	71/120	11/30	17/30	28/60	16/30	27/30	43/60
2	66/120	8/30	19/30	27/60	16/30	23/30	39/60
3	68/120	10/30	14/30	24/60	20/30	24/30	44/60
Total	205/360	29/90	50/90	79/180	52/90	74/90	126/180
Number of mussels parasitized by *E. inquilina*							
1	43/120	7/30	13/30	20/60	8/30	15/30	23/60
2	46/120	8/30	16/30	24/60	10/30	12/30	22/60
3	42/120	6/30	15/30	21/60	9/30	12/30	21/60
Total	131/360	21/90	44/90	65/180	27/90	39/90	66/180
Number of mussels parasitized by *U. cyprinae*							
1	16/120	2/30	3/30	5/60	3/30	8/30	11/60
2	14/120	0/30	3/30	3/60	2/30	9/30	11/60
3	12/120	0/30	2/30	2/60	4/30	6/30	10/60
Total	42/360	2/90	8/90	10/180	9/90	23/90	32/180
Number of mussels parasitized by *M. intestinalis*							
1	4/120	0/30	1/30	1/60	1/30	2/30	3/60
2	4/120	1/30	0/30	1/60	2/30	1/30	3/60
3	2/120	0/30	0/30	0/60	2/30	0/30	2/60
Total	10/360	1/90	1/90	2/180	5/90	3/90	8/180
Number of mussels parasitized by *P. maculatus*							
1	2/120	0/30	0/30	0/60	0/30	2/30	2/60
2	1/120	0/30	0/30	0/60	0/30	1/30	1/60
3	0/120	0/30	0/30	0/60	0/30	0/30	0/60
Total	3/360	0/90	0/90	0/180	0/90	3/90	3/180
Number of mussels parasitized by *S. mytilovum*							
1	3/120	1/60	0/30	1/30	0/30	2/30	2/60
2	4/120	2/60	0/30	2/30	0/30	2/30	2/60
3	5/120	2/60	0/30	2/30	0/30	3/30	3/60
Total	12/360	5/180	0/90	5/90	0/90	7/90	7/180
Number of mussels parasitized by *Martelia* spp.							
1	24/120	5/30	2/30	7/60	12/30	5/30	17/60
2	25/120	5/30	3/30	8/60	10/30	7/30	17/60
3	22/120	4/30	2/30	6/60	9/30	7/30	16/60
Total	71/360	14/90	7/90	21/180	31/90	19/90	50/180

ly in October, while the opposite trend was recorded for *Marteilia* spp. The results of the chi-square test indicated that the variable of time was significantly related to the prevalence of the above parasites (for *E. inquilina*: $\chi^2 = 14.700$, $P = 0.000$; for *U. cyprinae*: $\chi^2 = 10.782$, $P = 0.001$; for *Marteilia* spp.: $\chi^2 = 6.334$, $P = 0.012$).

DISCUSSION

In fish and shellfish farming, the choice of a sustainable farming system in a certain area depends on many parametres and, in most cases, the probability for development of various diseases is one. In the Thermaikos gulf, over 90% of mussel production in Greece takes place. The area is considered a relatively closed environment, rich in organic materials, as the fresh water from three major rivers is constantly mixed with the sea water (Krestenitis et al., 2012). Two production systems are used for th culture of the mussels in these, the 'long-line' and 'on-table'. Effect of these two systems in the prevalence of common parasites of Mediterranean mussels was investigated for the first time in Greece.

Compared to fish, in Mediterranean mussels fewer microorganisms cause diseases, most of them being parasites. Parasites identified in Mediterranean mussels in the present study have been previously reported in studies performed elsewhere (Rayyan and Chintiroglou, 2003; Rayyan et al., 2004; 2006; Francisco et al., 2010). In those studies, reported prevalence varied greatly, according to mussel size, season and culture site. In the present study, we selected sites with similar profiles and we sampled mussels of similar size were used, in order to minimize effects unrelated to the objective of the study. In addition, as observed prevalence of some parasites (e.g., *U. cyprinae*) may be influenced by the section of the vertical ropes from where sampling is performed, we only collected and studied mussels from the middle section of the ropes. It should also be noted that, in previous studies it has been found that observed prevalence of parasites of mussels depended on the method used to examine the mussels. For example, Thébault et al. (2005) used various techniques and found that observed prevalence of *Marteilia refringens* in oysters using *in situ* hybridization techniques was higher, than that found using only conventional histology. In the present study, histology was used to rapidly and simultaneously screen a large a number of mussels for the presence of many parasitic species. The method was applied across all samples collected during the study, which made the results of comparable value.

Selection of culture system may affect number of mussels infected by any parasite and intensity of parasitism. For example, Buck et al. (2005) found that blue mussels (*Mytilus edulis*) cultured in suspended offshore sites grew faster and better and were almost free of parasites compared to those cultured in suspended inshore sites. Those authors concluded that sea depth at which the mussels actually live, can influence the life cycle of some of their parasites and thus ultimately their parasitic load.

In our study, total number of mussels infected by any parasite was higher in mussels cultured with the 'on-table' system, compared to those cultured with the 'long-line' system. However, when each species of parasites was individually considered, infection by only two, namely *U. cyprinae* and *Marteilia* spp., of them had a significant relation. Low observed prevalence for *M. intestinalis*, *P. maculatus* and *S. mytilovum* infection did not allow a reliable statistical analysis. The low prevalence of infection by these parasites has been previously reported in other geographical areas of the world (e.g., Robledo et al., 1994a; Rayyan and Chintiroglou, 2003; Rayyan et al., 2004), although this can be influenced by many factors such as water pollution.

U. cyprinae is a free-living organism found on muddy bottoms (Marcus, 1951); very often, it inhabits the mantle cavity and particularly the gills of various bivalves, such as *Crassostrea virginica, Mytilus edulis, M. galloprovincialis* and *M. californianus* (Fleming et al., 1981; Fleming, 1986; Plourde et al., 1991; Bower et al., 1994; Caceres-Martinez et al., 1998). Increased prevalence of infection by this parasite in mussels cultured with the 'on-table' system, is in agreement with observations reported by Murina and Solonchenko (1991). Those authors noted that Mediterranean mussels living on silty sea beds had increased prevalence and intensity of infection by this parasite compared to those attached to ropes approximately 4 m above sea bottom. Life cycle of this parasite has not yet been fully elucidated, but it is believed that a part of it takes place at the bottom, where the cocoons are attached to various substrates (González et al., 2005). Thus, increased prevalence of infection noted in mussels cultured closer to the seabed ('on-table' system) can be explained as originating from free-swimming forms of parasites produced from the cocoons, which may

easier meet potential future hosts. Regarding the seasonal variation in the prevalence of infection by this parasite in Mediterranean mussels, reports are contradictory. We have observed increased prevalence at the end of the autumn compared to that recorded during the summer, which is in agreement with the findings of Robledo et al. (1994b), but differs from the reports of other authors (González et al., 2005; Mladineo et al., 2012), who found the opposite. The results can indicate that infection by this parasite may be dependent by various environmental parameters, which affect host-parasite relationship.

The results of the present study confirm those of Karagiannis and Angelidis (2007) in relation to infection by *Marteilia* spp. Although the complete life cycle of *Marteilia* spp. is still not fully elucidated, various studies have already identified some zooplankton species as likely intermediate hosts for the parasite (Carrasco et al., 2007). It appears that as these prefer estuarine waters rather than open seas, where the 'long-line' system is used, the possibility of infecting the mussels cultured with the 'on-table' system is higher. The findings of seasonal fluctuations of *Marteilia* spp. infection of the mussels are also in agreement with the study of Karagiannis and Angelidis (2007).

E. inquilina is, generally, considered to be a symbiont, rather than a parasite, which benefits from its host, in this case the Mediterranean mussel, without causing any harmful effect. Nevertheless, recent studies have suggested that this organism can adversely affect growth of infected mussels (Rayyan et al., 2004) and induce some histopathological changes, particularly in the mantle epithelium (Mladineo et al., 2012). The present study did not indicate any difference in the prevalence of the infection by this organism between the two culture systems, which suggests that the polyps can be found in the entire water column at many depths. For both culture systems, prevalence of the infection was higher in October compared to that in July, finding which is in agreement with earlier studies by Piraino et al. (1994), Rayyan et al. (2004) and (Mladineo et al., 2012). The increase in the prevalence of infection may be associated with the seasonality of the population of polyps observed in Thermaikos gulf, as Rayyan et al. (2004) have suggested.

CONCLUDING REMARKS

The study indicated that the system used for culture of Mediterranean mussels can affect prevalence of infection of some common parasites. In particular, number of mussels parasitized by any parasitic species was significantly increased in mussels cultured with the 'on-table' system compared to those cultured with the 'long-line' system. With regard to the prevalence of infection by specific parasites, that by *U. cyprinae* and *Marteilia* spp. was higher in mussels cultured with the 'on-table' system, while no difference for *E. inquilina* was noted between the two production systems.

CONFLICT OF INTEREST STATEMENT

The authors certify that there is no actual or potential conflict of interest in relation to this article.

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