

Integrated multi-trophic aquaculture of the European flat oyster (*Ostrea edulis* Linnaeus, 1758): A case study from Boka Kotorska Bay (Montenegro)

by

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Abstract

Although integrated multi-trophic aquaculture (IMTA) is recognized as a strategy to control and minimize the impact of fish farming on the marine environment, there are still many unknowns when it comes to this type of farming. This paper presents the results of research on the growth of European flat oysters in IMTA and monoculture systems. Growth was monitored at three different sites: near fish cages, 100 m from fish cages, and in a monoculture system, during an 18-month experiment. The highest mortality occurred at the site near the fish cages. At the end of the experiment, all monitored individuals reach commercial size, except for four individuals at the site near the fish cages. There were statistically significant differences in oyster growth with respect to site and period. At the site near the fish cages, oyster growth was significantly lower compared to the growth at the two other sites. The most intense growth of oysters occurred during the spring and early summer period. Our results indicate that the production cycle of oysters in integrated aquaculture and monoculture is quite similar and that sites directly adjacent to fish cages should be avoided for oyster farming.

Key words: Oysters, marine aquaculture, IMTA, Adriatic Sea

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1. Introduction

Marine aquaculture, specifically marine fish farming, is an important food sector that is typically carried out in sheltered coastal areas, particularly bays. In such areas, the marine environment may be greatly adversely impacted by eutrophication caused by the influx of organic matter originating from fish farms into the water column. Therefore, integrated multi-trophic aquaculture (IMTA) has been recognized as a good solution for overcoming the negative impact of fish farming. In addition to reducing the negative impact on the aquatic ecosystem, IMTA also implies diversification, i.e. introduction of new species into aquaculture (Troell et al. 2003; Barrington et al. 2009; Giangrande et al. 2020).

IMTA can be defined as the cultivation of fed aquaculture species, such as fish, in association with other species capable of extracting organic and/or inorganic matter from seawater, such as bivalves (Giangrande et al. 2020). Sabellids, sponges, cucumbers, polychaetes, and macroalgae are also recognized as very good candidates for IMTA (Giangrande et al. 2020; Nederlof et al. 2020; Grebe et al. 2021; Grosso et al. 2021). According to Cunha et al. (2019), integrated production of fish, oyster, phytoplankton, and macroalgae is an improved system compared to regular semi-intensive fish production. Although the benefits of IMTA are well known and academic attention has increased during the last two decades, this concept has not yet become a commercial reality in European marine aquaculture (Kleitou et al. 2018).

Since the eastern Adriatic Sea, including the Montenegrin coast, represents an oligotrophic environment (Tičina et al. 2020), marine aquaculture is only found in sheltered coastal areas that are mesotrophic or even eutrophic. One such area is Boka Kotorska Bay, the only sheltered area along the Montenegrin coast, where there are two fish and about 20 shellfish farms (Mandić et al. 2016; Gvozdenović et al. 2017; MONSTAT 2023). The Montenegrin marine aquaculture sector is stagnant despite the natural available potential, with 2021 production of 43 t of sea bass (*Dicentrarchus labrax*), 36 t of sea bream (*Sparus aurata*), 186 t of mussels (*Mytilus galloprovincialis*), and 32 t of oysters (*Ostrea edulis*) (MONSTAT 2023). Both fish farms are integrated farms, where fish and bivalves are farmed together.

In integrated bivalve–fish aquaculture, bivalves, as filter feeders, can remove organic particles as well as uneaten fish food from the water column (Redmond et al. 2010), thereby reducing eutrophication and increasing biomass production (Peharda et al. 2007). Consequently, native and commercially most important

bivalves, such as the Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) and the European flat oyster (*Ostrea edulis* Linnaeus, 1758), are good choices for integrated farming in the Mediterranean.

Currently, IMTA in the Mediterranean is only being developed on an experimental scale, and studies have yielded mixed results with regard to bivalve–fish integrated aquaculture. Some studies have suggested that bivalves are capable of feeding on particles derived from fish farms, which in turn enhances their growth rate (Mazzola & Sarà 2001; Lander et al. 2004; 2012; Peharda et al. 2007; Reid et al. 2010; MacDonald et al. 2011). Conversely, other studies have demonstrated negligible or even absent bivalve growth increase in fish farms (Cheshuk et al. 2003; Navarrete-Mier et al. 2010; Irisarri et al. 2015; Aguado-Giménez et al. 2014; Župan et al. 2016).

This article presents a pilot study of spatial and temporal differences in *O. edulis* growth in Boka Kotorska Bay, the only marine aquaculture area along the Montenegrin coast. The starting hypothesis was that oysters farmed in the proximity to fish cages consume organic waste originating from fish farms, which ultimately results in better growth. To investigate this hypothesis, the growth of oysters across three distinct sites was monitored: near fish cages, 100 m from fish cages, and in a monoculture system, for a period of 18 months.

2. Materials and methods

2.1. Study area

The study was conducted at two locations in Boka Kotorska Bay, Montenegro, in the southern Adriatic Sea (Fig. 1). One location was a fish and shellfish farm in the village of Orahovac, the municipality of Kotor (42°29'07.8"N; 18°44'42.36"E), while the other was the Sveta Nedjelja (Sv. Nedjelja) shellfish farm in the village of Kamenari, the municipality of Herceg Novi (42°27'30.96"N; 18°40'21.36"E). The straight-line distance between the two study locations is 8 km. The Orahovac farm is an integrated farm where *S. aurata*, *D. labrax*, *M. galloprovincialis*, and *O. edulis* are farmed together. Two sites on the Orahovac farm were selected for the experimental part of the study: the shellfish long-line closest to the floating fish cages (NBL), which was 10 m from the cages, and the shellfish long-line furthest (about 100 m) from the fish cages (NUD). The Sv. Nedjelja farm is a shellfish farm that produces *M. galloprovincialis*, as well as *O. edulis*, and therefore one site (SVN) was selected for the experiment on this farm.



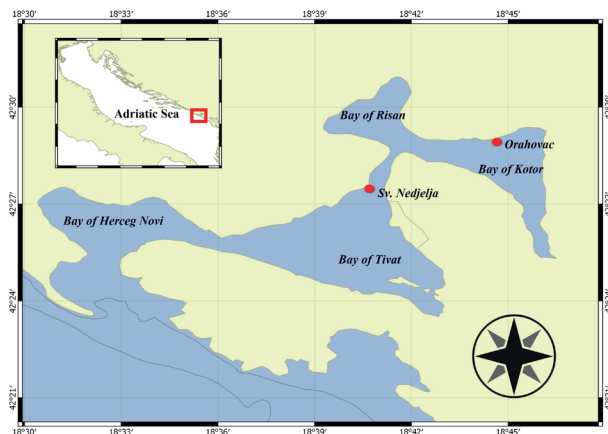


Figure 1

Location of the sampling sites (SVN at Sv. Nedjelja, NBL and NUD at Orahovac)

2.2. Experimental design

The growth experiment was conducted over a period of 18 months, from March 2015 to September 2016. In March 2015, oysters of a similar size (43.5 ± 6.5 mm) and age (approximately 13 to 15 months) were collected from the natural population in Boka Kotorska Bay and cleaned of fouling organisms. Vernier calipers were used to measure the shell length of each individual, with an accuracy of 0.1 mm.

All oysters were marked with improvised tags made of waterproof plastic mats designed for use in kitchens (WelkHOME, Italy). The mats were cut into rectangles measuring approximately 12×5 mm, and markings were etched onto the surface using a scalpel and soldering iron, with waterproof felt-tip markers used to enhance visibility. The numbered tags were attached to the oyster shells using a two-component waterproof adhesive (ABRO EPOXY STEEL, U.S.A.). During the tagging process, the oysters were exposed to air for approximately 2 to 3 min, until the adhesive hardened.

The marked and measured oysters were placed in plastic baskets ($48 \times 29 \times 5$ cm, with a stretched mesh size of 2 cm) and suspended in the water at a depth of 2 to 3 m in March 2015. A total of 110 individuals were placed at each site, resulting in 330 individuals in total. Every other month, the oysters were removed from the water, measured, cleaned of fouling organisms, and re-suspended in water. The oysters were measured on nine occasions: 1) March 2015 – May 2015; 2) May 2015 – July 2015; 3) July 2015 – September 2015; 4) September 2015 – November 2015; 5) November 2015 – January 2016; 6) January 2016 – March 2016; 7) March 2016 – May 2016; 8) May 2016 – July 2016; and 9) July 2016 – September 2016.

Temperature and salinity at both locations were measured monthly between March 2015 and September 2016, at a depth of 2 to 3 m, using a Multiline P4 WTW probe.

2.3. Data analysis

Microsoft Office Excel 2013 was used for descriptive statistics and data visualization. Repeated measures two-way ANOVA with Tukey post hoc test in the R Statistical Environment were used to test growth ratios. The parameters of the von Bertalanffy growth equation ($L_t = L_\infty [1 - e^{-k(t-t_0)}]$) were estimated using Munro's method from the FISAT II v.1.2.2 statistical package (Gayanilo et al. 2005), where L_t is the length of the oyster over time, L_∞ is the asymptotic size, and k is the growth coefficient.

3. Results

Temperature and salinity values of seawater are presented in Figures 2 and 3. The minimum temperature of 10.5°C was observed at Sv. Nedjelja in January 2016, while the maximum temperature of 27.9°C was recorded at Orahovac in July 2015. The average water temperature during the study period was 19.4°C and 19.3°C at Sv. Nedjelja and Orahovac, respectively. The salinity of water ranged from a minimum value of 15.5 PSU at Orahovac in May 2016 to a maximum value of 37.1 PSU at Sv. Nedjelja in December 2015. The average water salinity during the study period was 31.5 PSU and 27.8 PSU at Sv. Nedjelja and Orahovac, respectively.

A total of 169 individuals survived the entire experiment. The highest mortality rate was observed at the NBL site, with 56 individuals and an additional

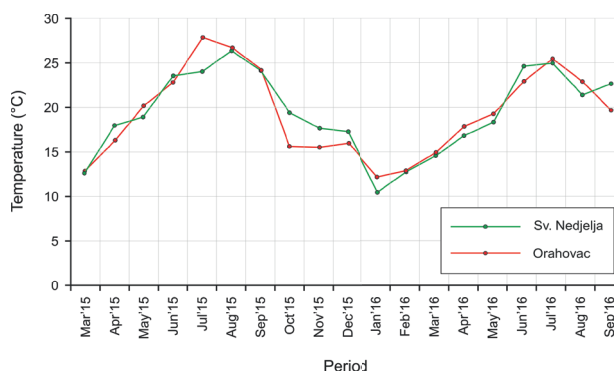


Figure 2

Monthly temperature values at both locations (Sv. Nedjelja and Orahovac) at a depth of 2 m to 3 m

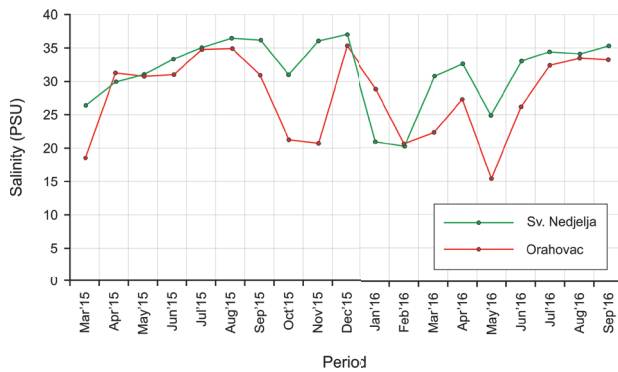


Figure 3

Monthly salinity values at both locations (Sv. Nedjelja and Orahovac) at a depth of 2 m to 3 m

14 individuals lost during the experiment. In total, 49 and 42 individuals died at the NUD and SVN sites, respectively. The highest mortality rate among all three sites was recorded during the third and fourth survey periods (July to November 2015). No mortality was recorded during the first and last survey periods. The mortality rate was higher in 2015 compared to 2016 (Table 1).

At the beginning of the experiment, oysters at all three sites had similar average lengths (Table 2). After 18 months, the average length of oysters at the NBL site was lower compared to NUD and SVN. The difference between the average length reached and

Table 1

Mortality in oysters by sampling period and site

Period	NBL	NUD	SVN
Mar'15-May'15	0	0	0
May'15-Jul'15	5	9	12
Jul'15-Sep'15	33	16	14
Sep'15-Nov'15	18	18	11
Nov'15-Jan'16	14*	3	3
Jan'16-Mar'16	0	2	1
Mar'16-May'16	0	1	0
May'16-Jul'16	0	0	1
Jul'16-Sep'16	0	0	0
Sum	70	49	42

*basket with 14 individuals lost

Table 2

Average oyster length and standard deviation per site, at the beginning and end of the experiment

Period	NBL	NUD	SVN
	mm		
Beginning (Mar'15)	43 ± 6.5	42.9 ± 6.5	44.6 ± 6.5
End (Sep'16)	75.4 ± 11	82.5 ± 9.3	85 ± 9
Difference	32.4 ± 11	39.5 ± 10.1	40.3 ± 10.4

the initial length was also the lowest at the NBL site. The average length reached, as well as the difference between length reached and initial length, between the NUD and SVN sites were similar.

After 18 months, all individuals at the NUD and SVN sites reached the commercial size of 60 mm in length, as defined by law (Official Gazette of Montenegro 65/2015), while four individuals at the NBL site did not reach the size. At the SVN site, 94% of the individuals reached the necessary commercial size after just 10 months, while at the NUD and NBL sites, 80% of the individuals reached the commercial size after the same period. Considering that the oysters were about 13 to 15 months old at the beginning of the experiment, it was estimated that the oyster production cycle was slightly shorter at the SVN site (23 to 25 months) compared to the NUD and NBL sites (25 to 27 months).

There were statistically significant differences in oyster growth according to site and period (Fig. 4; Table 3). Oysters from the NUD and SVN sites had similar growth rates, statistically higher compared to oysters from the NBL site. In terms of period, the highest growth rate was observed during the first period (March 2015 to May 2015), while the slowest growth was observed during the last two periods (May 2016 to July 2016 and July 2016 to September 2016).

Table 3

Analysis of oyster growth increments using repeated-measures ANOVA with Tukey post hoc comparison

Factor	d.f.	F	P	Post hoc comparison
Period	8	119.321	< 0.001	8 = 9 > 3 = 6 < 4 = 7 = 5 < 2 < 1 *
Site	2	7.536	< 0.001	NBL < NUD = SVN
Interaction	16	9.665	< 0.001	
Error	1494			

*1 – Mar'15-May'15; 2 – May'15-Jul'15; 3 – Jul'15-Sep'15; 4 – Sep'15-Nov'15; 5 – Nov'15-Jan'16; 6 – Jan'16-Mar'16; 7 – Mar'16-May'16; 8 – May'16-Jul'16; 9 – Jul'16-Sep'16

The lowest asymptotic length was recorded for oysters from the NBL site (77 mm), while oysters from the SVN and NUD sites had similar asymptotic lengths, 88.64 mm and 87.98 mm, respectively (Table 4).

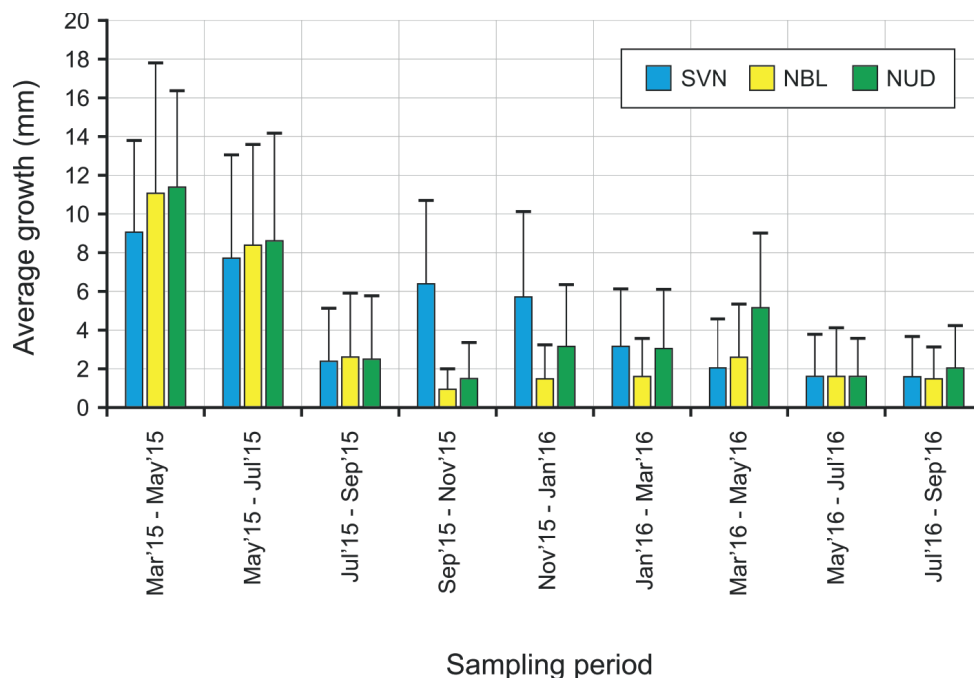
Table 4

Oyster growth parameters for each site

Parameter	NBL	NUD	SVN
L_{∞} (mm)*	77	87.98	88.64
k (year ⁻¹)**	1.965	1.676	2.101

* L_{∞} – asymptotic size; ** k – growth coefficient



**Figure 4**

Average oyster growth and standard deviation

4. Discussion

The implementation of IMTA using fish and bivalves has yielded contrasting results (Mazzola & Sarà 2001; Cheshuk et al. 2003; Lander et al. 2004; 2012; Peharda et al. 2007; Navarrete-Mier et al. 2010; Reid et al. 2010; MacDonald et al. 2011; Aguado-Giménez et al. 2014; Irisarri et al. 2015; Župan et al. 2016; Sanz-Lazaro & Sanchez-Jerez 2017). The present study demonstrated statistically significant spatial and temporal differences in the growth of oysters. Monoculture yielded better growth, while significantly lower growth rates were observed for oysters farmed near fish cages, consistent with the findings of Župan (2012) and Bajnoci (2014) for bivalve cultivation. Župan (2012) recommended avoiding sites near fish cages for bivalve farming due to the direct and intense impact from fish farming, while Sarà et al. (2009) reported that mussels grown near fish farms had higher growth rates compared to those grown far away. Sarà et al. (2012) demonstrated, however, through Dynamic Energy Budget modeling, that mussels and oysters farmed close to fish cages could reach a greater maximum length compared to those farmed far away. Lander et al. (2004) reported a 50% improvement in the growth of *Mytilus edulis* cultured next to *Salmo salar* farms. In contrast, Navarrete-Mier et al. (2010) and Irisarri et al. (2015) observed no differences in the growth of mussels and oysters farmed at varying distances from fish cages.

Furthermore, although Aguado-Giménez et al. (2014) showed that *O. edulis* cultured next to *S. aurata* and *D. labrax* farms in open-sea conditions use fish farm waste, the increase in growth was negligible. The above differences may result from differences in cultured fish and bivalve species, local primary production, as well as experimental designs (Peharda et al. 2007; Župan et al. 2014).

The oysters showed the most intense growth during the first period, in spring 2015. This observation is similar to that of Navarrete-Mier et al. (2010) in the western Mediterranean. There are several factors that affect the growth of bivalves, of which food supply is considered the most important. Since food supply depends on temperature, salinity, depth, and other factors, it is challenging to quantify the exact effect of a single environmental factor on the growth of bivalves (Gosling 2003). The position of the ropes on the rafts and the position of the bivalves relative to currents are also factors that can affect the growth of bivalves (Peharda et al. 2007). Since spring and summer are the periods of the highest primary production in Boka Kotorska Bay, as well as the period when temperatures rise, it can be concluded that an adequate food supply and favorable temperatures contributed to the higher oyster growth during spring and early summer in this study. It is important to note that Boka Kotorska Bay is a ROFI (Region of Freshwater Influence) due to the presence of rivers, streams, submarine springs, high

precipitation, all of which enrich the bay with nutrients, leading to phytoplankton growth (Bellafiore et al. 2011; Drakulović et al. 2011; 2012; 2017).

When comparing oyster growth across sampling periods, it is evident that growth dynamics varied by location. During the first three sampling periods, oysters from the IMTA sites (NUD and NBL) exhibited more intense growth compared to oysters in monoculture (SVN). However, during the next three sampling periods, intense growth of oysters from the SVN site was observed compared to oysters from the NUD and NBL sites (Fig. 4). Aguado-Giménez et al. (2014) observed similar differences in growth dynamics of flat oysters between IMTA and reference locations in the western Mediterranean, while Handā et al. (2012) found that fish farming contributed to higher growth rate of bivalves only at certain times of the year. Although the observed pattern of growth dynamics in our study suggests that during the first three study periods the oysters at the IMTA sites were most likely feeding on organic matter originating from fish farms, further research effort should focus on verifying this fact, as the observed results in growth rates do not necessarily prove the assimilation of fish farming waste. The observed differences in growth may also be due to a general difference in trophic condition between the sites, not just the presence of fish farms.

The highest oyster mortality at the site adjacent to fish cages, as reported in this study, has also been observed at other Mediterranean farms (Sarà et al. 2007). A thick fouling community was observed during the fieldwork at this site throughout the study period, leading to conclusion that these two aspects are interdependent. A dense fouling community can cause growth stagnation and higher mortality rates in flat oysters (Stjepčević 1974). The highest mortality observed in the summer and early autumn of 2015 in this study coincides with the period of highest temperature and salinity. Higher mortality of flat oysters in summer has been reported along the Adriatic Sea (Zrnčić et al. 2007), in other Mediterranean areas (Carlucci et al. 2010), and along the coasts of the United Kingdom and France (Askew 1972; Robert et al. 1991). Stjepčević (1974) indicated that unfavorable environmental conditions, such as high temperature and salinity, are devastating to flat oysters.

Mandić & Huter (2014) reported that the production cycle of flat oysters in Boka Kotorska Bay is 24 to 30 months, a timeframe that also applies to flat oysters farmed along the Croatian coast (Radetić 2010; Zelić 2015). The results obtained in this study concerning the production cycles of flat oysters (23 to 25 months at SVN; 25 to 27 months at NUD and NBL sites) are consistent with the literature data mentioned above.

5. Conclusions

The results of this study make a valuable contribution to the understanding of IMTA farming of flat oysters in coastal marine aquaculture. One of the major challenges of IMTA is to comprehend the flow and dynamics of organic particles originating from fish farms, which are highly dependent on physical, chemical, and biological parameters. This is crucial because filter feeders can effectively feed on these particles and reduce their negative impact on the marine environment and further increase the productivity of the entire system. Based on the findings of this study, it is apparent that further research is necessary in this area. Therefore, there are plans to significantly expand research on this topic.

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