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PRIMARY RESEARCH PAPER

Growth of *Mytilus galloprovincialis* (mollusca, bivalvia) close to fish farms: a case of integrated multi-trophic aquaculture within the Tyrrhenian Sea

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Abstract A current practice of marine aquaculture is to integrate fish with low-trophic-level organisms (e.g. molluscs and/or algae) during farming to minimise effects of cultivation on the surrounding environment and to potentially increase economic income. This hypothesis has been tested in the present article experimentally, by co-cultivating fish and mussels (*Mytilus galloprovincialis*) in the field. Integrated multi-trophic aquaculture (IMTA) experiments were started in July 2004 by transplanting mussel seed at two depths (−3 and −9 m) within 1,000 m downstream to fish cages and at 1,000 m upstream from cages. Mussels were cultured in nylon net bags for 12 months and the growth recorded biometrically. The outcome of our field experiment corroborated the idea of IMTA effectiveness. In fact, in the study area, the organic matter from fish-farm biodeposition caused changes in the chemical environment (i.e. controls and impacted sites were significantly different for organic matter availability and chlorophyll-*a*) and this induced changes in growth performance of co-cultivated mussels. Mussels cultivated close to cages, under direct organic emission, reached a higher total length, weight and biomass than mussel cultivated far from farms.

Keywords *Mytilus galloprovincialis* · Fish farm · Organic enrichment · Integrated multi-trophic aquaculture (IMTA) · Fish · Mediterranean

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Introduction

A current practice of marine aquaculture is to integrate fish with low-trophic-level organisms (e.g. molluscs and/or algae) during farming to minimise effects of cultivation on the surrounding environment and to potentially increase economic income. Troell et al. (2003) have reviewed the research on integrated aquaculture (viz. integrated multi-trophic aquaculture—IMTA) in the literature, summarising its strength and weakness and identifying future directions research in this area should take. In particular, the value of IMTA has been emphasised as an ecological engineering practice to limit the environmental impact of waste from fish cultivation (through recycling of particulate and dissolved matters) and for enhancing the total productivity (in weight and in value) (Troell et al., 2003). Since large aggregations of aquatic organisms, such as cultivated fish and shrimps, produce large quantities of allochthonous dissolved and particulate organic matter (Modica

et al., 2006; Sarà et al., 2006; Sarà, 2007a, b, c), the secondary co-cultivated low-trophic-level species (molluscs or algae) would allow recycling of this allochthonous waste (i.e. fish; Troell & Norberg, 1998). Thus, energy contained in the waste that otherwise would be lost to the surrounding environment, is transformed into edible biomass and channelled through the adjacent trophic webs as evidenced by some studies using the stable isotope approach (Mazzola & Sarà, 2001; Gao et al., 2006, 2008). Feed that is otherwise lost and spread out in the environment would result in organic accumulation on sediments (Kalantzi & Karakassis, 2006) and in the water column (Modica et al., 2006; Sarà et al., 2006; Sarà, 2007b). IMTA originates from freshwater practices and was only recently initiated in marine ecosystems (e.g. Neori et al., 1991; Lin et al., 1993; Shpigel et al., 1993a, b; Troell et al., 2003). The most persuasive data indicating benefits of this type of practice have come from mesocosm or enclosure experiments (e.g. Jones et al., 2001; Neori et al., 2004). Although, the enclosure experiments appeared to result in a consistent reduction of negative impacts (Jones & Preston, 1999), their applicability is rather limited due to experimental conditions (Sarà, 2007a). However, there is renewed interest in IMTA as a tool for reducing effluents (sensu Lindahl et al., 2005) and there are many suggestions for integrated cultivation systems using different combinations of seaweeds, bivalves, fish and shrimps. IMTA is not employed widely in the Mediterranean, perhaps for economic and social reasons (Sarà, 2007a, b, c), and this region was selected for study. The main aims of the present article was to verify if the growth performance of mussels (*Mytilus galloprovincialis*) cultivated with fish were different, over time, as compared to mussels cultivated in control areas (no fish facilities).

Materials and methods

The study area

The experimental study of integrated cultivation was carried out during 2004 off the northern coast of Sicily, in the Gulf of Castellammare (South Tyrrhenian). Five submersible cages (Farmocean, Sweden; volume = 4,500 m^3; 12-m deep) and six smaller cages (volume = 1,000 m^3; 6-m deep) were deployed in the eastern part of the Gulf (Latitude 38°04′53″N; Longitude 13°02′04″E) and moored on the bottom at a depth of about 32 m, about 0.7 nm off the coast (Fig. 1). The hydrodynamic regime of the cage area was characterised by a dominant current coming from the third and fourth quadrants (along a west-east axis) for most of the year (Modica et al.,

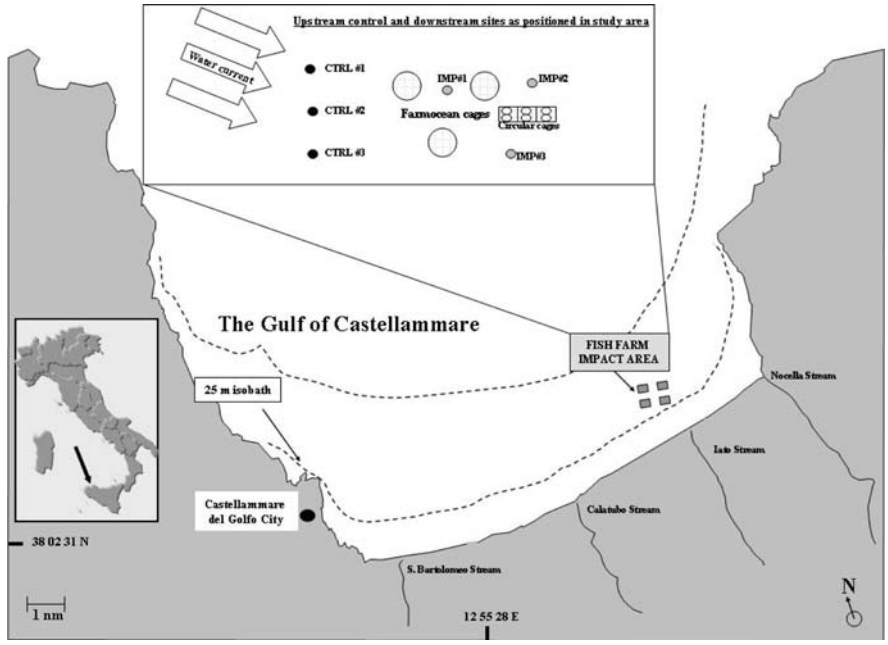

Fig. 1 Map of the study area, the Gulf of Castellammare (Sicily, MED), showing the area (detail in the upside not has a scale) of the fish cages and control sites

2006; Sarà et al., 2006, 2007). The cages were filled with seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*) for a total annual production of about 600 tons of biomass. Throughout the farming period, the average food conversion ratio was roughly estimated by the aquaculture owners to be about 1.9 on average for both species and the total supplied feed (different types of commercial pelleted feed produced by BioMar, France and Hendrix, Italy) was over 1,000 tons. During the experiment the total biomass was roughly always the same in all the cages.

Experimental design, sampling and laboratory analysis

To address the question of whether organic discharge coming from aquaculture facilities had an effect on the growth performances of integrated low-trophic-level organisms, we verified possible trophic differences among sites due to cage influence. Effects were evaluated between downstream cage sites (within 1,000 m downstream from cages; hereafter IMP) and sites far from cages ($\sim$1,000 m upstream; hereafter CTRL) using chlorophyll-*a* and suspended and particulate organic matter descriptors. Accordingly, in June 2004 and May 2005, water samples were collected from IMP and CTRL sites from 3 and 10 m using Niskin bottles and were analysed for chlorophyll-*a*, total suspended matter (organic + inorganic fraction by loss on ignition) and particulate organic matter as expressed by the sum of proteins, lipids and carbohydrates (see Sarà et al., 2006 for details on methods).

IMTA experiments started in July 2004 by transplanting a total of 240 nylon bags containing each about 5.6 ($\pm$0.36) kg of seed of *M. galloprovincialis* (from Northern Adriatic hatcheries), which were attached to buoys and positioned within 1,000 m downstream from cages (impact) along the main water current direction (Sarà et al., 2006) and upstream (controls; 1,000 m far from fish cages) at two depths (-3 and -9 m) in each site. Thus, we transplanted 20 bags for each depth, in each site ($n = 3$) of each IMP and CTRL location. Mussels were cultured for 12 months. Two groups of about 120 specimens were bimonthly collected by scuba divers from each depth at both CTRL and IMP sites and were analysed in the laboratory through biometrical and gravimetrical measurements (Sarà et al., 1998). All specimens from each sample were measured to the nearest 0.1 mm with a digital calliper to determine total shell length (TL; mm) and total thickness (TT; mm), while the total wet weight (TW; g) of individuals was measured to the nearest 0.1 g. Somatic tissue and valves from each specimen were excised, dried separately at 105°C ($\sim$48 h), and combusted at 500°C for 24 h to obtain the somatic and valve ash free dry weight (S-AFDW and V-AFDW, respectively; g) to the nearest 0.1 g. The sum of somatic and valve AFDW values were used in this article as an estimate of secondary production (Weinberg, 1978; Lucas, 1993; Sarà et al., 2007).

Statistical analysis

An analysis of variance (ANOVA; Underwood, 1997) was carried out to test for differences in suspended matter, chlorophyll-*a* and particulate organic matter concentrations between CTRL and IMP sites. During this analysis, Condition (DISTURB; DI, two levels; control and impact), periods (PERIOD; PER, two levels; June 2004 and May 2005) and depth (DEPTH; DE; -3 and -10 m) were chosen as fixed factors, and three sites were treated as random and nested in Condition. Three replicates were collected for each of the following variables: total suspended matter, chlorophyll-*a* and particulate organic matter features (lipids, proteins and carbohydrates). To test the difference in biometry and production of mussels cultivated under control and impact conditions, a three-way ANOVA was used. For the biometrical variables, DISTURB (DI, two levels; control and impact), and PERIOD (PE, six levels; START [transplantation time, July 2004 = Period 1], September = Period 2, December = Period 3, March = Period 4, June = Period 5 and early August 2005 = Period 6) and DEPTH (DE, two levels; -3 vs. -9 m) were treated as fixed factors. Different sites (Site, three levels) were treated as random factors and were nested in DISTURB. In the case of biomass, the experimental design was the same except for the sites. Two sampling replicates ($n = 2$) were chosen randomly for each period and site. The heterogeneity of variances was tested using Cochran's *C* test prior to the analysis of variance, and Student–Newman–Keuls (SNK) test allowed the appropriate means comparison. Growth performances

were studied by analysing the relationship between the total shell length (TL) and total wet weight (TW) using a simple allometric equation ($TW = a\ TL^b$; Gould, 1966) and logarithmic transformations (White, 2003). The analysis of covariance (ANCOVA, Underwood, 1997) was used to test the heterogeneity of slopes and differences between intercepts of allometric regressions for comparing the annual growth between the populations cultivated under different conditions. The GMAV statistical package (Institute of Marine Ecology, University of Sydney, Sydney) was used to perform the ANOVA, and Microsoft Excel was used to perform ANCOVA.

Results

Impacted and control sites significantly differed as chlorophyll-*a*, total suspended matter and particulate proteins were significantly higher close to the cages than far from them (ANOVA, $P < 0.05$; Table 1). No difference between depths was detected (ANOVA, $P > 0.05$). Mussels were transplanted in the summer 2004 and had an initial average total length of 26.6 ± 1.5 mm and weight of 1.3 ± 0.1 g (Fig. 2, Table 2). After 1 year of cultivation, the size of mussels was significantly different among controls and impacts throughout the study periods. In particular, control and impact mussels were different in their length in periods 3, 5 and 6 (Fig. 2). Thickness differed significantly between control and impact sites in periods 3, 5 and 6 like length (figure not reported) while control and impact differences in wet weight and total ash free dry weight (AFDW; Table 3) were detected only in periods 5 and 6 (Figs. 3, 4). Length–weight relationships (Table 4) were highly significant. Differences in annual total allometry between CTRL and IMP was significant (ANCOVA, $P < 0.05$). Mussels cultivated in IMP sites showed a decline of allometric ratios in the spring while in control sites mussels showed a general and continual decrease in allometric coefficients from

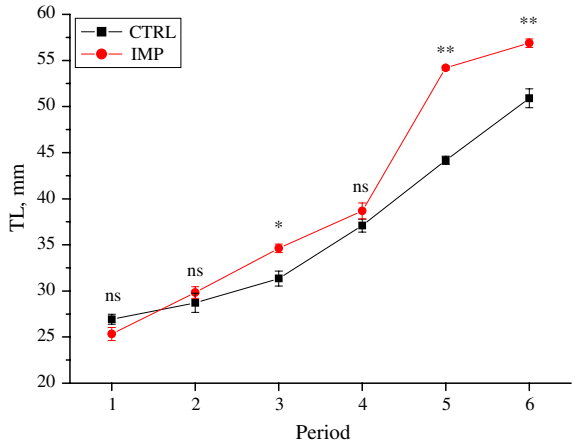

Fig. 2 Total length (TL, mm) comparing controls (CTRL) and impact (IMP) throughout the study period (statistical notation over each point deals with comparison of control vs. impact in every period, * $P < 0.05$; ** $P < 0.01$; *ns* not significant; please note that since there was not significant differences between depths, values were pooled; see Table 2)

Table 1 Trophic variables measured in control and impact locations of the study area in summer 2004 and spring 2005 and ANOVA results testing difference between control and impact sites, while depths not significant different throughout the analysed periods

	CTRL			IMP		*P*
	Mean	±SE		Mean	±SE	CTRL vs. IMP
Total suspended matter, mg l^{-1}	8.1	1.3	>	7.7	1.2	*
Total suspended organic matter, mg l^{-1}	1.2	0.2	<	2.1	0.8	**
Chlorophyll-*a*, μg l^{-1}	1.1	0.7	<	1.7	0.5	**
Phaeopigments, μg l^{-1}	3.3	2.7	<	4.6	1.6	*
Particulate carbohydrates, μg l^{-1}	69.8	21.3	>	39.1	4.7	**
Particulate proteins, μg l^{-1}	132.4	31.0	<	158.1	39.1	*
Particulate lipids, μg l^{-1}	33.8	4.5	<	68.6	13.8	*
Particulate organic matter (POM), μg l^{-1}	235.9	52.2	<	265.7	38.1	*
Proteins: carbohydrates	1.9	0.5	<	4.0	0.7	**

CTRL control, *IMP* impact, ±*SE* standard error, *P* probability level, *ns* not significant difference
* $P < 0.05$, ** $P < 0.01$

Table 2 ANOVA performed on total length (TT), total thickness (TT) and total weight (TW)

Source	df	TL			TT			TW		
		MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
DISTURB (DI)	1	415.8	39.96	*	23.34	41.87	*	12.15	117.99	**
Site (DI)	2	10.4	4.97	**	0.56	1.54	ns	0.10	0.29	ns
DEPTH (DE)	2	15.17	0.85	ns	0.33	0.24	ns	0.15	0.16	ns
PERIOD (PE)	5	2973.25	370.09	***	483.75	521.92	***	182.95	383.48	***
DI × DE	2	16.74	0.93	ns	0.88	0.65	ns	0.33	0.37	ns
DI × PE	5	100.72	12.54	***	7.96	8.59	***	6.20	12.99	***
DE × Site (DI)	4	17.93	8.57	***	1.36	3.74	**	0.89	2.53	ns
DE × PE	10	10.88	1.07	ns	0.87	0.76	ns	0.78	1.11	ns
PE × Site(DI)	10	8.03	3.84	***	0.93	2.55	**	0.48	1.36	ns
DI × DE × PE	10	6.86	0.68	ns	1.04	0.91	ns	0.50	0.71	ns
PE × DE × Site (DI)	20	10.14	4.85	***	1.15	3.16	***	0.70	2.01	*
Residuals	72	2.09			0.36			0.35		
Cochran's *C*				ns			ns			ns

ns not significant difference

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 3 ANOVA performed on ash free dry weight (AFDW) data

Source	df	MS	*F*	*P*
DISTURB (DI)	1	38.32	8.91	***
DEPTH (DE)	1	12.02	2.80	ns
PERIOD (PE)	5	644.73	149.97	***
DI × DE	1	0.15	0.03	ns
DI × PE	5	32.36	7.53	***
DE × PE	5	2.40	0.56	ns
DI × DE × PE	5	6.56	1.53	ns
Residuals	24	4.30		
Cochran's *C*	47			ns

ns not significant difference

*** $P < 0.001$

the transplantation period until the end of cultivation (Table 4).

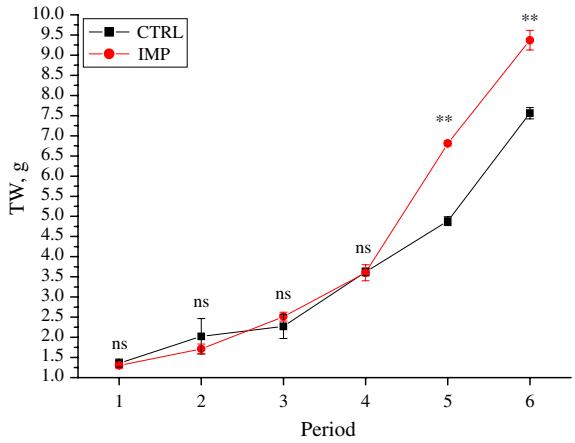

Fig. 3 Total weight (TW, g) comparing controls (CTRL) and impact (IMP) throughout the study period (statistical notation over each point deals with comparison of control vs. impact in every period, * $P < 0.05$; ** $P < 0.01$; *ns* not significant; please note that since there was not significant differences between depths, values were pooled; see Table 2)

Discussion

IMTA is often cited as a practice that increases the environmental sustainability of aquaculture and economic income (Troell et al., 2003, Neori et al., 2004). In land-based systems, 100% nutrient removal can be achieved (ammonium) and energy contained in organic discharge can be efficiently transformed by associated low-trophic-level species. Other studies have shown the effectiveness of IMTA for salmon co-cultured with bivalves (Stirling & Okumus, 1995; Cheshuk et al., 2003), algae with bivalves (Qian et al., 1996) and echinoderms with abalones (Kang et al., 2003). The outcome of our field experiment corroborated the idea of IMTA for caged finfish and co-cultured bivalves. Previous studies carried out in

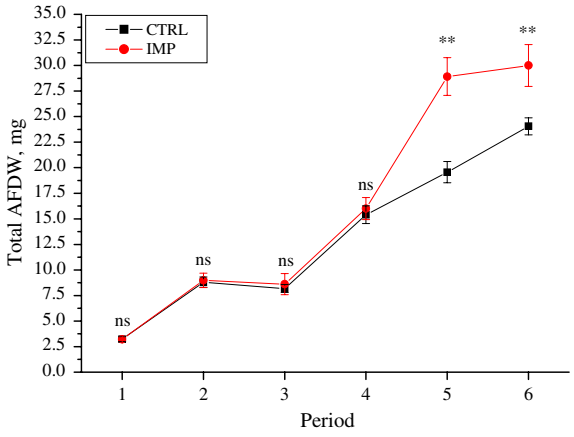

Fig. 4 Total ash free dry weight (soma + valves) comparing controls (CTRL) and impact (IMP) throughout the study period (statistical notation over each point deals with comparison of control vs. impact in every period, * $P < 0.05$; ** $P < 0.01$; *ns* not significant; please note that since there was not significant differences between depths, values were pooled; see Table 2)

the same area showed that fish-farming waste represents a primary source of organic matter (Sarà et al., 2006), which is diluted in the water column and is potentially available to suspension feeders cultivated around the fish cages. The input of organic matter from farms can be considered quantitatively quite constant throughout the year (Mazzola & Sarà, 2001), while phytoplankton and terrigenous-continental organic carbons represent secondary highly variable sources (Modica et al., 2006; Sarà et al., 2006). The present study shows that the organic matter from fish-farm biodeposition can cause changes in the chemical environment because controls and impacts in the study area differed significantly in terms of available organic matter for secondary consumers, similar to changes observed in other Mediterranean areas (Pitta et al., 1999, 2005; see the review on this topic of Sarà, 2007b). This may induce changes in growth performance of co-cultivated mussels due to their filtration capacity (Lindahl et al., 2005). Mussels cultivated close to cages, reached a higher total length, wet and ash free weight than mussels cultivated far from farms. These results are in agreement with those obtained by other authors. For example, experiments on fish-mussel IMTA (Stirling & Okumus, 1995; Cheshuk et al., 2003) showed an effect produced by different cultivation conditions, and in our experiment these effects were amplified (see Table 4 reported in Sarà et al., 1998 for comparison with other study cases). Previous experiments carried out in the same oligotrophic area but in the absence of fish facilities (Sarà & Mazzola, 1997; Sarà et al., 1998) highlighted that the natural trophic availability of the Gulf waters sustained the secondary production of transplanted mussels. Nevertheless, in those experimental conditions, organic matter

Table 4 Allometric relationships between total length (TL) and total wet weight (TW) at each depth

Site	Period	Depth (m)	*n*	*a*	±	*b*	±	*r*	Depth (m)	*n*	*a*	±	*b*	±	*r*
CTRL	1	3	1,739	−8.76	0.07	2.69	0.02	0.95	9	1,739	−8.76	0.07	2.69	0.02	0.95
CTRL	2	3	743	−9.22	0.10	2.83	0.03	0.96	9	617	−8.74	0.09	2.69	0.03	0.97
CTRL	3	3	422	−8.77	0.12	2.70	0.04	0.96	9	339	−8.20	0.24	2.54	0.07	0.89
CTRL	4	3	357	−7.57	0.20	2.45	0.06	0.92	9	67	−8.20	0.57	2.60	0.16	0.90
CTRL	5	3	205	−7.79	0.22	2.46	0.06	0.95	9	181	−7.01	0.33	2.24	0.09	0.88
CTRL	6	3	143	−5.48	0.33	1.93	0.09	0.88	9	129	−8.13	0.43	2.53	0.11	0.90
CTRL	Year	3	2,381	−9.25	0.06	2.86	0.02	0.96	9	1,770	−8.78	0.06	2.70	0.02	0.96
IMP	1	3	1,739	−8.76	0.07	2.69	0.02	0.95	9	1,739	−8.76	0.07	2.69	0.02	0.95
IMP	2	3	406	−9.20	0.16	2.85	0.05	0.95	9	578	−9.22	0.07	2.81	0.02	0.98
IMP	3	3	166	−9.11	0.30	2.82	0.08	0.93	9	189	−8.69	0.22	2.66	0.06	0.95
IMP	4	3	168	−6.13	0.47	1.98	0.13	0.77	9	179	−5.72	0.35	1.92	0.10	0.83
IMP	5	3	172	−7.75	0.21	2.42	0.05	0.96	9	162	−7.60	0.24	2.37	0.06	0.95
IMP	6	3	122	−8.84	0.72	2.73	0.18	0.81	9	120	−7.77	0.29	2.45	0.07	0.95
IMP	Year	3	1,641	−8.63	0.05	2.67	0.02	0.97	9	1,641	−8.94	0.05	2.73	0.01	0.98

n sample size, *a* log of intercept, ± standard errors for intercepts and/or for slopes, *b* slopes of allometric regressions, *r* correlation coefficient

availability was not sufficient to elicit high levels of gonadal output for maintaining stable populations over time as deduced also by the fact that there was no mussel seed in the vicinity of facilities (Sarà et al., 1998). Instead, the flux of organic particles from cages would induce spawning in most of individuals as might be evidenced by the presence of high densities of juvenile mussels in the fouling of farms (Sarà et al., 2007). Mussels also exploited organic particles coming from facilities showing that the flux of organic emission could be efficiently transformed into secondary bivalve biomass co-cultured with fish. This result is also corroborated by data on fouling reported in a recent paper (Sarà et al., 2007): fouling abundance and biomass was higher closer to cages than at far sites. Moreover, our results suggest that fish farm organic waste that is dispersed in the water column may be a food source for bivalve molluscs such as mussels. As filter feeders, they are essentially generalist consumers of POM (Dame, 1996) and exploit organic matter from several sources (autochthonous, terrigenous natural allochthonous or anthropogenic) as a function of its availability (Langdon & Newell, 1990; Stirling & Okumus, 1995; Riera & Richard, 1997; Sarà et al., 1998, 2003; Lindahl et al., 2005). The direct use of waste organic matter by bivalves would reduce the environmental impact of organic waste from fish-farming activities like in other environmental situations (e.g. Swedish coasts; Lindahl et al., 2005). Bivalves functioning as recyclers of allochthonous organic matter could contribute to environmentally clean aquaculture (Jones & Iwama, 1991; Shpigel et al., 1993a, b; Lefebvre et al., 2000; Mazzola & Sarà, 2001) and could increase the profitability of fish cultivation.

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