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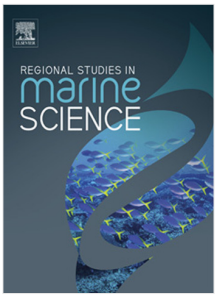

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Differences in magnitude and spatial extent of impact of tuna farming on benthic macroinvertebrate assemblages

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Abstract

Differences in magnitude and spatial extent of impact of three tuna farms located in Malta on polychaete and amphipod assemblages associated with soft sediment habitat were assessed using a hierarchical spatial design that incorporated different spatial scales, from tens of meters to a few kilometers. Spatial variation in impact was significant at the scale of location, at which farm size and local environmental factors differed. The magnitude of impact was higher at the larger farm, as indicated by elevated levels of sediment fish bone content, significantly lower number of polychaete families, and the

'Poor' ecological quality status recorded for the seabed area occupied by the cages. The influence of tuna farming activities on the benthic macroinvertebrate assemblages extended up to c. 1 km away from the cages, possibly due to transportation of particulate organic waste there via sea currents.

Keywords: Mediterranean Sea; Tuna farming; Environmental impact; Aquaculture; Benthic assemblages; Spatial variability[1]

1. Introduction

Farming of Atlantic bluefin tuna (*Thunnus thynnus thynnus* Linnaeus 1758) is a large sector of the aquaculture industry, which however has raised concerns on sustainability (see review by Metian et al., 2014). Atlantic Bluefin Tuna (ABT) is captured in the Mediterranean from the wild and transferred to cages for fattening (FAO, 2005-2011) using whole bait fish as feed (Aguado et al., 2004; Vita and Marin, 2007). The uneaten feed-fish that accumulate below the tuna cages are the main source of pollution of the seabed (Aguado-Giménez et al., 2006; Mangion et al., 2014; Vita and Marin, 2007). The

1 After (Af); Atlantic Bluefin Tuna (ABT); Before/After (BA); Before (Be); Biota and/or environment matching (BIOENV); Ecological Quality Status (EQS); Impacted (Im); Location (Lo); Northeastern farm (NEF); Number of families (NoF); Percent feed-fish bone content (PFBC); Percent organic carbon content (POCC); Percent organic nitrogen content (PONC); Permutational analysis of variance (PERMANOVA); Plot (Pl); BOPA Fish farming (BOPA-FF) index; Reference (Re); Shannon Wiener diversity (ShW); Site (Si); Southeastern Farm 1 (SEF 1); Southeastern Farm 2 (SEF 2)

tuna are farmed at high stocking densities, which entail high feed input; however, these vary between different farms. As a result, one would expect differences in the level of adverse environmental impact, when present, between different farms. Potential adverse impacts of tuna farming on the seabed may be reduced or eliminated when the cages are located in exposed sites characterised by deep waters, where strong bottom currents prevail (Maldonado et al., 2005).

Several studies have addressed the environmental effects of tuna farming in the Mediterranean, including the potential adverse effects of ABT farming on nutrient levels in the water column and sediment (Aksu et al., 2010; Dal Zotto et al., 2016; Marin et al., 2007; Matijević et al., 2006, 2008; Vita et al, 2004; Vita and Marin, 2007; Vezzulli et al., 2008), and microbial levels in the water column (Kapetanović et al., 2013). Other studies assessed the indirect effects of the ABT penning industry via the use of diesel fuel (Hospido and Tyedmers, 2005), impact of ABT farming on *Posidonia oceanica* meadows (Kružić et al., 2014), wild fish assemblages associated with the tuna pens (Šegvić Bubić et al., 2011), and effects of ABT farming on trophic food-web linkages (Forrestal et al., 2012). Several studies on the influence of the activity on benthic macroinvertebrate assemblages in the vicinity of the tuna pens have also been published (Jahani et al., 2012; Mangion et al., 2014, in press; Marin et al., 2007; Moraitis et al., 2013; Vezzulli et al., 2008; Vita and Marin, 2007). A comparison of the benthic impacts of ABT farming with those of other Mediterranean farming activities, namely sea bass and sea bream rearing, is available in San-Lázaro and Marin (2008).

Different conclusions have been reached on the level and spatial extent of adverse effects of fish farming on the seabed because the experimental design, method, and indicators used, as well as local environmental factors, vary widely between different study sites (Kalantzi and Karakassis, 2006). To properly address the environmental impact of ABT farming on benthic habitat, it is desirable to include multiple spatial scales in the sampling design (Wiens, 1989). Determination of appropriate spatial scales at which potential environmental impacts of aquaculture may be investigated is necessary to enable proper assessment of patterns of variation in the influence of the activity on the marine environment (Fernandez-Gonzalez et al., 2013). Several studies have assessed patterns of variation in the influence of fish farming on benthic habitat at a number of spatial scales (e.g. Gyllenhammar and Håkanson, 2005; Fernandez-Gonzalez et al., 2013), but in the case of tuna farming this aspect has not been given sufficient attention (but see Moraitis et al., 2013; Vita and Marin, 2007).

The use of polychaetes (e.g. Aguado-Giménez et al., 2015; Mangion et al., in press; Martinez-Garcia et al., 2013; Sutherland et al., 2007; Tomassetti and Porrello, 2005) and amphipods (e.g. Fernandez-Gonzalez et al., 2013; Fernandez-Gonzalez and Sanchez-Jerez, 2011; Mangion et al., in press) as biological indicators of fish farming impacts on benthic habitat is well known. The polychaete/amphipod (BOPA) ratio is a benthic index developed for the European Water Framework Directive (WFD, 2000/60/EC) (Dauvin and Ruellet, 2007; Gomez-Gesteira and Dauvin, 2000), that has also been used to classify coastal waters under the influence of fish farming activities (e.g. Aguado-Giménez et al., 2015; Jahani et al., 2012; Mangion et al., in press) into ‘High’, ‘Good’, ‘Moderate’,

'Poor', or 'Bad' Ecological Quality Status (EQS) classes (Dauvin and Ruellet, 2007; Gomez-Gesteira and Dauvin, 2000). The BOPA index uses frequency data and the proportion of organisms in each category, which render it independent of sampling protocols that utilize different mesh sizes and measurements used to express the abundance of organisms per unit area. Another major advantage of the BOPA index is the reduced taxonomic effort required to assess the ecological quality status (EQS) of the marine environment. The BOPA index has been applied to measure the impact of various environmental disturbances, and has been shown to be effective in detecting the presence of hydrocarbons (Dauvin and Ruellet, 2007; Gomez-Gesteira and Dauvin, 2000) and sewage discharges (de-la-Ossa-Carretero et al., 2009) in certain zones, such as oyster culture areas (Bouchet and Sauriau, 2008) and harbors (Ingole et al., 2009). However, BOPA tends to overestimate the EQS compared to other benthic indices (see de-la-Ossa-Carretero and Dauvin, 2010). A modification of the BOPA index was proposed by Aguado-Giménez et al. (2015) to improve its performance in Mediterranean areas affected by fish farming activities.

The main aim of the present study was to assess the magnitude and spatial extent of tuna farming on soft bottom polychaete and amphipod assemblages using a hierarchical spatial design; from tens of meters to a few kilometers, using abundance of three selected indicator taxa, total number of taxa and Shannon-Wiener diversity of the polychaete and amphipod taxa, and the fish farm polychaete/amphipod index (as modified by Aguado-Giménez et al., 2015). In the present study, the hypothesis that particulate organic matter originating from the tuna cages and settling to the seabed leads to changes in the

invertebrate assemblages associated with the soft bottom habitat was tested using data on sediment physico-chemical attributes, namely w/w feed-fish bone content (PFBC); which represents the uneaten feed-fish that decomposed on the seabed; mean sediment grain size (MSGS), percent organic carbon content (POCC), and percent organic nitrogen content (PONC). Three tuna farms located in the Maltese Islands and differing in size, stocking density and feed management regime, were used in the present assessment.

2. Materials and Methods

2.1. Study sites and sampling

The three Maltese tuna farms considered in the present study are located 1 km offshore (Figure 1) where the seabed consists of soft sediment. One farm is located off the northeastern coast, where water depth is some 45 m – 50 m, while the other two farms are located off the southeastern coast where water depth is some 42 m – 53 m, and are some 1.5 km apart. The northeastern farm (NEF) had eight tuna cages having a maximum total annual capacity of 2500 t, while the two southeastern farms were smaller (maximum total annual capacity of 1500 t each); one having three cages (southeastern Farm 1 [SEF 1]) and the other (southeastern Farm 2 [SEF 2]) having four cages (ICCAT, 2011). All three farms utilize cages having a diameter of some 50 m and a height of around 25 m. The tuna stocking density was circa 100 $\pm$ 200 t per cage, and the fish were fed the equivalent of 3-4% of the fish biomass per day, divided over two feeding sessions (tuna farm

managers, personal communication). The feed consisted of whole bait fish; namely mackerel, sardines, squid and prawn; and the ratio of food (based on the wet weight of the feed) that is converted to tuna biomass is around 10-15:1 (tuna farm managers, personal communication). However, the feeding regime is expected to differ between the different farms as a result of adaptive management to natural environmental factors (e.g. sea current strength), and depending on the growth rate of the tuna.

The sampling design incorporated three fixed, orthogonal, factors: (a) Before/After (BA), with two sampling periods, (i) in November 2000 at NEF, in October 2002 at SEF 1, and in June 2001 at SEF 2 'before' initiation of the tuna farming activities, and (ii) in November 2001 at NEF, in October 2003 at SEF 1, and in June 2002 at SEF 2, 'after' initiation of the activity; (b) Location (Lo), with three farms (i) NEF, (ii) SEF 1, and (iii) SEF 2; and (c) Plot (Pl), measuring some 300 m by 500 m, with two treatments: (i) 'impacted' plot; i.e. the seabed area where the tuna cages were sited, and (ii) 'reference' plot, located some 1 km – 1.5 km away from the cages. A random factor 'Site' (Si) was nested within the 'BA x Lo x Pl' interaction, with sites separated at the scale of hundreds of meters. Three sampling sites were allotted to each level of the three-way interaction, as the minimum number of cages at any one of the farms was three, such that a total of eighteen sampling sites are included in the sampling design.

Sampling was carried out using a 0.1 m^2 van Veen grab. Three replicate grab samples for benthic macrofaunal studies and one grab sample for sediment physico-chemical studies were collected at each of the eighteen sampling sites. The collected samples were live-

sieved (0.5 mm mesh) on board the vessel and afterward temporarily preserved in 10% formalin.

In the laboratory, samples for faunal studies were sorted for polychaetes and amphipods after washing on a 0.5 mm mesh. Specimens were identified to the family level (see Karakassis and Hatziyanni, 2000; Olsgard and Somerfield, 2000) and enumerated to obtain estimates of number of families and abundance per grab sample. For sediment physico-chemical studies, sub-samples were frozen at -20°C for later analyses to determine the POCC, PONC and PFBC, while another sub-sample was oven dried for granulometric analysis. Analysis of the sediment to determine the PFBC was carried out by sorting fish bones from the sediment using forceps under a dissecting microscope. POCC in the sediment was determined by wet oxidation using a chromic acid-sulfuric acid mixture, and titration of the evolved carbon dioxide (see Walkley and Black, 1934). PONC in the sediment was determined by the Kjeldhal method, i.e. by digestion in concentrated sulfuric acid containing a copper sulfate catalyst, addition of excess strong alkali, and condensation of the ammonia given off for titration. Measurement of MSGS was carried out according to Buchanan (1984) (see Holme and McIntyre, 1984).

Unpublished data on sea current direction and velocity collected every three months during the period 2010 to 2017 at the northeastern and southeastern farm sites at water depths of between 1 m and 10 m, using drogues according to the Lagrange method, were obtained from Ecoserv Ltd.

2.2. Data analyses

Indicator taxa at family level were selected as the three most abundant (in terms of number of individuals) macroinvertebrates (see Morrisey et al., 1992) before tuna farming activities were initiated. The polychaete/amphipod (BOPA-Fish farming [BOPA-FF]) index was calculated using BOPA = log ((f_P / f_A +1) +1); where 'f_P' is the frequency of polychaetes tolerant to organic enrichment resulting from fish farming activities, as identified by Martinez-Garcia et al. (2013) (see Aguado-Giménez et al., 2015), and 'f_A' is the frequency of amphipod individuals excluding the genus *Jassa* (Dauvin and Ruellet, 2007). Boundary values between 'High' ($0.00 \geq x > 0.09$), 'Good' ($0.09 \geq x > 0.16$), 'Moderate' ($0.16 \geq x > 0.25$), 'Poor' ($0.25 \geq x > 0.30$), and 'Bad' (≥ 0.30) EQS classes are as given in Dauvin and Ruellet (2007).

Four-factor univariate permutational analysis of variance (PERMANOVA) (Anderson, 2001) was used (with α set at 0.05) on a Euclidean similarity matrix to test the hypothesis of no difference in tuna farming activities between different farms in terms of (i) abundance of selected indicator taxa Maldanidae, Paraonidae and Glyceridae (polychaetes), and of Lysianassidae, Phoxocephalidae and Urothoidae (amphipods), (ii) number and Shannon-Wiener diversity of polychaete and amphipod families (Morrisey et al., 1992), and (iii) polychaete/amphipod (BOPA-FF) index as defined by Aguado-Giménez et al. (2015). Separate univariate PERMANOVA was carried out (with α set at 0.05) using a Euclidean similarity matrix to test the hypothesis of no difference in tuna farming activities between different farms in terms of the sediment MSGS, POCC and

PONC, using a model with three fixed, orthogonal factors 'BA', 'Lo' and 'Pl', and treating the levels of 'Si' as replicates. When the PERMANOVA indicated a significant difference, the source of significant difference was identified for the highest interaction term using *a posteriori* pair-wise tests. To determine which sediment physico-chemical variable, or combination of variables, best explained the observed variation in the macroinvertebrate assemblages, the BEST routine of the biota and/or environment matching (BIOENV) analysis (Clarke and Gorley, 2006) was carried out, using the Spearman rank correlation method and D1 Euclidean similarity measure, at the level of the 2-way interaction terms, as the number of replicates at the level of 'BA x Lo x Pl' was too low. All the analyses were implemented using PRIMER v.7.0.11 (PRIMER software; Clarke and Gorley, 2006) and the PERMANOVA+ v.1.0 add-on package (Anderson et al., 2008).

3. Results

3.1. Macroinvertebrate data

A total of 5,750 individuals from 26 polychaete families, and 2,103 individuals from 22 amphipod families, were collected. The top families (in terms of number of individuals) that characterised the polychaete and amphipod assemblages at the three tuna farms before farming activities commenced were: Maldanidae, Paraonidae and Glyceridae (polychaetes), and Lysianassidae, Phoxocephalidae and Urothoidae (amphipods) (Figure 2).

PERMANOVA indicated no significant difference for the interaction term 'BA x Lo x Pl' in the abundance of polychaetes and amphipods, while 'BA x Lo' was significant for abundance of Glyceridae ($p < 0.05$) and Urothoidae ($p < 0.01$), 'BA x Pl' was significant for abundance of Urothoidae ($p < 0.001$), and 'Pl x Lo' was significant for abundance of Maldanidae ($p < 0.05$), Glyceridae ($p < 0.01$), Urothoidae ($p < 0.001$) and Phoxocephalidae ($p < 0.01$) (Table 1). Pair-wise tests showed that the abundance of Glyceridae recorded from the NEF impacted/reference plots increased significantly ($p < 0.05$) following the tuna farming activities, while the abundance of Urothoidae ($p < 0.01$) and Phoxocephalidae ($p < 0.001$) was significantly low at the NEF impacted plot compared to the NEF reference plot before/after the tuna farming activities (Table 1, Figure 2). At the southeastern farms, the abundance of Urothoidae recorded from the impacted/reference plots decreased significantly ($p_{SEF\ 1} < 0.05$, $p_{SEF\ 2} < 0.001$) following the tuna farming activities. The abundance of Phoxocephalidae was significantly high ($p < 0.05$) at the SEF 1 impacted plot compared to the SEF 1 reference plot, while the abundance of Glyceridae ($p < 0.05$), Urothoidae ($p < 0.05$) and Phoxocephalidae ($p < 0.001$) was significantly high at the SEF 2 impacted plot compared to the SEF 2 reference plot, before/after the tuna farming activities (Table 1, Figure 2).

PERMANOVA indicated a significant difference in the abundance of Lysianassidae for 'BA' ($p < 0.05$), and in the abundance of Paraonidae for 'Lo' ($p < 0.05$) (Table 1). Pair-wise tests showed that the overall abundance of Lysianassidae decreased significantly ($p < 0.05$) following initiation of tuna farming, while the overall abundance of Paraonidae was significantly high ($p < 0.05$) at SEF 1 compared to NEF and SEF 2 (Table 1, Figure

2). PERMANOVA also indicated a significant difference in the abundance of Maldanidae ($p < 0.001$), Paraonidae ($p < 0.05$), Lysianassidae ($p < 0.05$) and Urothoidae ($p < 0.01$) for 'Si(BA x Lo x Pl)' (Table 1).

PERMANOVA indicated a significant difference in the Shannon-Wiener diversity of polychaetes ($p < 0.01$), number of amphipod families ($p < 0.05$), and Shannon-Wiener diversity of amphipods ($p < 0.001$) for the interaction term 'BA x Lo x Pl' (Table 1). Pair-wise tests showed that, following initiation of the tuna farming activities, the Shannon-Wiener diversity of polychaetes ($p < 0.001$), number of amphipod families ($p < 0.01$), and Shannon-Wiener diversity of amphipods ($p < 0.01$) recorded at the NEF impacted plot, decreased significantly (Table 1, Figure 3). The number of amphipod families at the SEF 2 impacted ($p < 0.05$) and reference plots ($p < 0.01$), and the Shannon-Wiener diversity of amphipods at the SEF 2 reference plot ($p < 0.05$), decreased significantly in the same period (Table 1, Figure 3).

PERMANOVA indicated significant differences in the number of polychaete families for 'BA' ($p < 0.05$), 'Lo' ($p < 0.001$) and 'Pl' ($p < 0.01$) (Table 1). Pair-wise tests showed that the overall number of polychaete families decreased significantly ($p < 0.05$) following the tuna farming activities, and was significantly low ($p < 0.001$) at NEF compared to SEF 1 and SEF 2, and at the impacted plots compared to the reference plots ($p < 0.01$) (Table 1, Figure 3). PERMANOVA also indicated a significant difference ($p < 0.05$) in the number of families and Shannon-Wiener diversity of polychaetes for 'Si(BA x Lo x Pl)' (Table 1).

PERMANOVA indicated a significant difference in BOPA-FF for the interaction term 'BA x Lo x Pl' ($p < 0.01$) (Table 1). Values of the mean BOPA-FF index indicated 'High' EQS at NEF, 'Good'/'High' EQS at SEF 1, and 'High' EQS at SEF 2, at the impacted and reference plots prior to the initiation of tuna farming activities (Figure 3), and pair-wise tests indicated no significant difference in BOPA-FF in that period (Table 1). Following initiation of tuna farming activities, BOPA-FF increased significantly at the NEF ($p < 0.01$) and SEF 2 ($p < 0.05$) at the impacted plots. The mean EQS was 'Poor' at the NEF impacted plot and 'High' at the NEF reference plot, while the pair-wise tests showed that BOPA-FF was significantly high ($p < 0.001$) at the NEF impacted plot compared to the NEF reference plot in the same period. There was no significant difference in BOPA-FF between the mean 'Moderate' and 'Good' EQS recorded respectively at the SEF 1 impacted and reference plots following tuna farming, nor between the mean 'Good' and 'Moderate' EQS recorded respectively at the SEF 2 impacted and reference plots in the same period. Pair-wise tests showed that BOPA-FF was significantly high ($p_{SEF\ 1} < 0.05$, $p_{SEF\ 2} < 0.001$) at the NEF impacted plot compared to the two southeastern farms' impacted plots following the farming activities. No significant difference in BOPA-FF was detected between the two southeastern farms' impacted plots in the same period (Table 1, Figure 3).

3.2. Sediment physico-chemical data

The sediment PFBC recorded below fish cages following the tuna farming activities was higher at the NEF (2.33% ± 3.12%) compared to the two southeastern farms, and higher at SEF 1 (1.59% ± 2.66%) compared to SEF 2 (0.04% ± 0.06%).

PERMANOVA indicated a significant difference in sediment POCC and PONC for 'BA x Lo x Pl' ($p < 0.05$) (Table 2). Pair-wise tests showed that, following initiation of tuna farming, POCC increased significantly ($p < 0.05$) at the NEF impacted plot (Table 2). The general increase in POCC recorded at the SEF 1 impacted plot, and at the SEF 2 reference plot (Figure 4), was not significant. PONC increased significantly ($p < 0.05$) at the SEF 1 reference plot following the tuna farming activities, and was significantly high ($p < 0.05$) at the SEF 1 reference plot compared to the NEF reference plot in the same period (Table 2). A general increase in PONC following tuna farming activities was observed at the NEF impacted plot (Figure 4), while no significant difference was detected for this sediment attribute at NEF from before to after initiation of tuna farming, nor between the impacted and reference plot afterwards (Table 2).

The general trend in MSGS was similar before and after the tuna farming activities, at the impacted and reference plots of each of the three tuna farms (Figure 4), with no significant difference indicated for 'BA', 'Lo', and 'Pl'; and interactions terms (Table 2).

3.3. Relationship between macroinvertebrates and sediment physico-chemical attributes

BEST analysis showed that a combination of MSGS and POCC was significantly correlated with the Shannon-Wiener diversity of polychaetes that was recorded overall during the study period at the NEF impacted plot ($\rho = 0.607$, $p < 0.05$), and with the Shannon-Wiener diversity of polychaetes ($\rho = 0.668$, $p < 0.05$), number of amphipod families ($\rho = 0.613$, $p < 0.05$), and Shannon-Wiener diversity of amphipods ($\rho = 0.810$, $p < 0.01$) recorded overall at the NEF impacted and reference plots after the tuna farming activities (Table 3).

At SEF 1, a significant correlation was recorded between POCC and number of polychaete families ($\rho = 0.852$, $p < 0.05$), and between a combination of POCC and PONC, and Shannon-Wiener diversity of polychaetes ($\rho = 0.921$, $p < 0.001$) recorded from the impacted plot before/after the tuna farming activities. BEST analysis also showed significant correlation between POCC, and abundance of Lysianassidae ($\rho = 0.815$, $p < 0.05$) and Shannon-Wiener diversity of amphipods ($\rho = 0.871$, $p < 0.01$) recorded overall from the SEF 1 impacted/reference plots after tuna farming; between a combination of MSGS and POCC, and abundance of Glyceridae ($\rho = 0.604$, $p < 0.05$) and the BOPA-FF index ($\rho = 0.754$, $p < 0.05$) recorded from the SEF 1 reference plot before/after the tuna farming activities; and between PONC, and abundance of Glyceridae recorded overall from the SEF 1 impacted/reference plots before initiation of tuna farming ($\rho = 0.931$, $p < 0.01$) (Table 3).

At SEF 2, a significant correlation was recorded between PONC and abundance of Paraonidae ($\rho = 0.671$, $p < 0.05$) recorded from the impacted plot before/after tuna farming; between a combination of MSGS and POCC, and abundance of Lysianassidae ($\rho = 0.865$, $p < 0.05$), and POCC and abundance of Urothoidae ($\rho = 0.832$, $p < 0.05$), recorded from the reference plot before/after the tuna farming activities; and between MSGS and abundance of Phoxocephalidae ($\rho = 0.766$, $p < 0.05$) recorded from the impacted/reference plots before the tuna farming activities (Table 3).

4. Discussion

The present results show that tuna farming activities resulted in alterations to the benthic invertebrate assemblages via accumulation of uneaten feed-fish on the seabed below the tuna cages. Values of the biological attributes assessed in the present work varied spatially, particularly at the scale of location (km). Previous studies at Mediterranean fish farms recorded high spatial variation in attributes of peracarid crustacean assemblages in the vicinity of fish cages (Fernandez-Gonzalez et al., 2013; Fernandez-Gonzalez and Sanchez-Jerez, 2011). Consideration of spatial variation in ecological studies that utilise a hierarchical nested design is important since the power of statistical tests is reduced (see Morrisey, 1992) when small scale variation is larger than the variation at higher spatial scales (e.g. Anderson et al., 2005; Chapman et al. 2010; Fernandez-Gonzalez et al., 2013; Fraschetti et al., 2005). In the present hierarchical study design, the power of statistical tests to detect observed differences in attributes of the benthic assemblage was increased

(see Morrisey, 1992) by setting location as a fixed factor, rather than as a random factor nested within the higher scale of impacted/reference plot.

Studies at other Mediterranean tuna farms reported low diversity of benthic assemblages below fish cages during the farming season (Jahani et al., 2012; Mangion et al., 2014; Marin et al., 2007; Vita and Marin, 2007), and elevated values of the ratio polychaete/amphipod abundance (BOPA) (Jahani et al., 2012). However, Moraitis et al. (2013) found no significant influence of tuna farming on benthic assemblages in Greece, which was attributed to exposure, hence to a high energy environment that helped dispersal of organic matter generated at the farm. The effects of fish farm wastes on seabed habitats are determined by local environmental characteristics, such as bottom type, water depth, exposure, and bottom currents, as well as the farms' feed management regime (Borja et al., 2009; Tomassetti et al., 2009). Therefore, differences in the level and spatial extent of potential adverse environmental impacts of tuna farming are expected between sites having different environmental characteristics. The three tuna farms investigated in the present study differed in size, stocking density, and feed management, as well as in their location; hence one would expect differences in the magnitude and spatial extent of potential adverse environmental impact among them.

Spatial variation in the influence of tuna farming on the polychaete and amphipod assemblages was significant at the scale of location. Furthermore, the number of polychaete families was significantly lower, and values of the polychaete/amphipod ratio were significantly higher at the impacted plot of the northeastern farm, where a 'Poor'

EQS was recorded. Concomitantly, the sediment POCC increased significantly at the impacted plot, while levels of sediment fish bone content below fish cages at the northeastern farm were elevated compared to the southeastern farms. In the present study, the northeastern farm has the largest annual fish holding capacity compared to the other two farms. Borja et al. (2009) previously reported that benthic ecological quality was better at fish farm sites that had a lower total annual production, which is in agreement with the present results. The sediment MSGS and POCC were significantly correlated with the diversity of polychaete families, and with the number and diversity of amphipod families recorded overall at the impacted plot of the northeastern farm, and at the northeastern farm after the tuna farming activities.

The influence of tuna farming on benthic habitat at the impacted plots of the southeastern farms was indicated by a significant decrease in the number of amphipod families, and the significant influence of sediment POCC and PONC, on the abundance and diversity of polychaete and amphipod families. The elevated levels of sediment PFBC below the tuna cages of southeastern Farm 1 compared to southeastern Farm 2, and the 'Moderate' EQS recorded from the impacted plot of southeastern Farm 1, indicate that the influence of tuna farming on benthic habitat present in the immediate vicinity of southeastern Farm 2, which retained 'Good' EQS, was not as large.

The level of tuna farming activities and feed management regime adopted at different tuna farms resulted in different levels of impact on sediment quality between cages within the same farm (Mangion et al., 2014), over and above the expected variation

between different tuna farms. Given potential high variation in biological attributes at small spatial scales, the pattern of influence of a fish farm on benthic biota at one site cannot be extrapolated to other farms at different sites (e.g. Fernandez-Gonzalez et al., 2013). Fernandez-Gonzalez et al. (2013) noted that spatial variation in attributes of benthic assemblages between different sites may be higher at fish farms compared to reference areas; this is characteristic of stressed assemblages (e.g. Stark et al., 2003; Warwick and Clarke, 1993). For instance, for the same farm considered in the present study, i.e. the northeastern farm, Mangion et al. (2014) reported a significantly higher abundance of Capitellid polychaetes below cages, which varied at the scale of site. The present results showed that, when considering the three tuna farms, significant variation in the abundance of polychaetes (Maldanidae, Paraonidae) and amphipods (Lysianassidae, Urothoidae), number of polychaete families, and Shannon-Wiener diversity of polychaetes was recorded at the scale of 'site', i.e. 100's of meters.

The spatial extent of influence of fish farm waste on the marine environment will vary (Karakassis et al., 2005) from a localised level to a regional one that may extend several kilometres (Silvert, 1992). The influence of tuna farming on benthic habitat detected in the present study appears to exceed the largest spatial scale incorporated in the survey design, since some influence of the activity on macroinvertebrate assemblages was detected c. 1 km away from the cages. Fernandez-Gonzalez et al. (2013) reported an influence of fish farming on spatial patterns of attributes of amphipod assemblages at spatial scales that varied from several meters to hundreds of kilometers. While a distance of c. 1 km would appear to be sufficient to minimize the influence of fish farm wastes on

a reference area (Porello et al., 2005), the oligotrophic nature of the Mediterranean may render the benthic ecosystem more sensitive to the organic input. Present results indicate that tuna farming at southeastern Farm 2 resulted in a significant decrease in the number of families and diversity of amphipods, and in a 'Moderate' EQS, at the reference plot located c. 1 km away from the tuna cages. The orientation of the reference plot of southeastern Farm 2 with respect to both impacted plots of the two southeastern farms, may account for the influence of tuna farming observed there, since organic waste may have been transported to the reference plot via sea currents; the acquired sea current data indicated a predominantly southern current (189 ^{o}) having a mean velocity of 0.185 ms^{-1} in the vicinity of the two southeastern farms. It is possible that other unidentified factors apart from the tuna farming activities may be influencing the soft bottom habitat at the reference plot of southeastern Farm 2, although the changes recorded there are in all probability due to the tuna farming activities, since they coincide with the onset of tuna farming in the general area. Apart from the location and size of the farm, the magnitude and spatial extent of tuna farming is also determined by a farm's specific feed management regime (Mangion et al., 2014).

5. Conclusion

The present results show that the magnitude of influence of tuna farming activities on benthic invertebrate assemblages varies significantly among different tuna farming locations having different farm sizes and local environmental and oceanographic factors. The influence of tuna farming activities on benthic invertebrate assemblages was larger at

the impacted plot of the largest tuna farm - in terms of ABT holding capacity and production - compared to the other two smaller farms. On the other hand, the spatial extent of impact appeared to be largest at one of the southeastern farms (Farm 2), where the influence of tuna farming activities extended down-current in a southerly direction, up to some 1 km away from fish cages; this may possibly reflect an 'additive effect' of the two southeastern farms, given that they are relatively close to each other (1 km apart). Taken together, these observations corroborate the expectation that the level and extent of influence of tuna farming activities on benthic habitat in the vicinity will be larger for farms having higher fish stocking density. Furthermore, farms located relatively close to one another may result in added loading on the environment, resulting in larger spatial extent of environmental impact - this latter observation has implications for spatial planning of tuna farming activities, particularly given that many countries are moving toward establishing 'allocated zones for aquaculture' (AZA); see Sanchez-Jerez et al. (2016). Finally, the present findings also show that inclusion of multiple reference areas in monitoring programmes is important for assessing potential environmental impacts of tuna farms.

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Figures

Fig. 1 Location of Malta at the centre of the Mediterranean (a); and map of the Maltese Islands showing the locations of the northeastern farm (NEF), southeastern Farm 1 (SEF 1), and southeastern Farm 2 (SEF 2) (b). Im = impacted plot; Re = reference plot

Fig. 2 Mean values (± SE) per grab of the number of individuals of Polychaete indicator taxon Maldanidae (a), Paraonidae (b), Glyceridae (c), and Amphipod indicator taxon Lysianassidae (d), Urothoidae (e), Phoxocephalidae (f), recorded before (black bars) and after (white bars) tuna-penning activities at the impacted (Im) and reference (Re) plots of

the northeastern farm (NEF), southeastern Farm 1 (SEF 1) and southeastern Farm 2 (SEF 2).

Fig. 3 Mean values (± SE) per grab of the total number of families (a, c) and Shannon-Wiener diversity (b, d) of polychaetes (a, b) and amphipods (c, d), and the polychaete/amphipod ratio (e) recorded before (black bars) and after (white bars) tuna-penning activities at the impacted (Im) and reference (Re) plots of the northeastern farm (NEF), southeastern Farm 1 (SEF 1) and southeastern Farm 2 (SEF 2).

Fig. 4 Mean values (± SE) per grab of sediment mean grain size (a), percent organic carbon content (b), and percent organic nitrogen content (c) recorded before (black bars) and after (white bars) tuna-penning activities at the impacted (Im) and reference (Re) plots of the northeastern farm (NEF), southeastern Farm 1 (SEF 1) and southeastern Farm 2 (SEF 2).

Table 1 Results of the 4-factor PERMANOVA for number of individuals of selected indicator taxa, number of families, and Shannon-Wiener diversity of polychaetes and amphipods, and the polychaete/amphipod ratio. Level of significance set at 0.05. Key: Degrees of freedom (df), Number of individuals (NI), Polychaete indicator taxon Maldanidae (1), Paraonidae (2), Glyceridae (3), Amphipod indicator taxon Lysianassidae (1), Urothoidae (2) and Phoxocephalidae (3), Number of families (NoF), Shannon-Wiener diversity (ShW), BOPA-Fish farming index (BOPA-FF), Before (Be), After (Af), Impacted plot (Im), Reference plot (Re), Northeastern farm (NEF), Southeastern Farm 1 (SEF 1), Southeastern Farm 2 (SEF 2), Not significant (ns), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

			Polychaeta					Amphipods					
Source of Variation		df	NI 1	NI 2	NI 3	NoF	ShW	NI 1	NI 2	NI 3	NoF	ShW	BOPA-FF
Before/After = BA		1	ns	ns	ns	*	***	*	***	**	***	***	***
Location = Lo		2	*	ns	ns	**	***	ns	***	ns	***	***	**
Plot = Pl		1	***	*	***	***	***	ns	ns	**	***	***	ns
BA x Lo		2	ns	ns	ns	ns	*	ns	ns	**	***	***	***
BA x Pl		1	ns	ns	*	ns	**	ns	ns	***	ns	ns	ns
Pl x Lo		2	*	ns	**	ns	***	ns	**	***	***	***	***
BA x Lo x Pl		2	ns	ns	ns	ns	**	ns	ns	ns	*	***	**
Site = Si (BA x Pl x Lo)		24	***	*	ns	*	*	*	**	ns	ns	ns	ns
RES		72											
TOT		107											
Pair-wise tests for the 3-way interaction term 'BA x Lo x Pl'													
NEF	Im		-	-	-	-	> ***	-	-	-	< **	< **	< **
	Re		-	-	-	-	ns	-	-	-	ns	ns	ns
SEF 1	Im		-	-	-	-	ns	-	-	-	ns	ns	ns
	Re	Be, Af	-	-	-	-	ns	-	-	-	ns	ns	ns
SEF 2	Im		-	-	-	-	ns	-	-	-	> *	ns	< *
	Re		-	-	-	-	ns	-	-	-	> **	> **	ns
Be	NEF		-	-	-	-	< *	-	-	-	> **	> *	ns
	SEF 1	Im, Re	-	-	-	-	ns	-	-	-	ns	ns	ns
	SEF 2		-	-	-	-	ns	-	-	-	ns	ns	ns

Af		NEF	-	-	-	-	< ***	-	-	-	> **	> ***	> ***
		SEF 1	-	-	-	-	ns	-	-	-	ns	> *	ns
		SEF 2	-	-	-	-	ns	-	-	-	ns	ns	ns

Table 1 Continued

			Polychaeta					Amphipods					
			NI 1	NI 2	NI 3	NoF	ShW	NI 1	NI 2	NI 3	NoF	ShW	BOPA-FF
Pair-wise tests for the 3-way interaction term 'BA x Lo x Pl'													
Be	Im	NEF, SEF 1	-	-	-	-	ns	-	-	-	< *	ns	ns
		NEF, SEF 2	-	-	-	-	< **	-	-	-	< **	< **	ns
		SEF 1, SEF 2	-	-	-	-	ns	-	-	-	< *	< **	ns
	Re	NEF, SEF 1	-	-	-	-	ns	-	-	-	ns	ns	ns
		NEF, SEF 2	-	-	-	-	ns	-	-	-	ns	< **	ns
		SEF 1, SEF 2	-	-	-	-	ns	-	-	-	ns	< *	ns
Af	Im	NEF, SEF 1	-	-	-	-	< **	-	-	-	< **	< **	> *
		NEF, SEF 2	-	-	-	-	< ***	-	-	-	< *	< **	> ***
		SEF 1, SEF 2	-	-	-	-	ns	-	-	-	ns	ns	ns
	Re	NEF, SEF 1	-	-	-	-	ns	-	-	-	ns	ns	< *
		NEF, SEF 2	-	-	-	-	ns	-	-	-	> *	> *	ns
		SEF 1, SEF 2	-	-	-	-	ns	-	-	-	> **	> *	ns
Pair-wise tests for the 2-way interaction terms 'BA x Lo', 'BA x Pl' and 'Pl x Lo'													
NEF			-	-	*	-	-	-	-	ns	-	-	-
SEF 1	Be, Af		-	-	ns	-	-	-	-	> *	-	-	-
SEF 2			-	-	ns	-	-	-	-	> ***	-	-	-
Be	NEF, SEF 1		-	-	< ***	-	-	-	-	ns	-	-	-
	NEF, SEF 2		-	-	< ***	-	-	-	-	< ***	-	-	-
	SEF 1, SEF 2		-	-	ns	-	-	-	-	ns	-	-	-
Af	NEF, SEF 1		-	-	< ***	-	-	-	-	> *	-	-	-
	NEF, SEF 2		-	-	ns	-	-	-	-	ns	-	-	-
	SEF 1, SEF 2		-	-	> ***	-	-	-	-	ns	-	-	-
Im	Be, Af		-	-	-	-	-	-	-	> ***	-	-	-
Re			-	-	-	-	-	-	-	ns	-	-	-
Be	Im, Re		-	-	-	-	-	-	-	> ***	-	-	-
Af			-	-	-	-	-	-	-	< *	-	-	-
Im	NEF, SEF 1		< **	-	< ***	-	-	-	ns	< **	-	-	-
	NEF, SEF 2		ns	-	< ***	-	-	-	ns	< ***	-	-	-
	SEF 1, SEF 2		ns	-	ns	-	-	-	ns	ns	-	-	-
Re	NEF, SEF 1		< *	-	< ***	-	-	-	> **	> **	-	-	-
	NEF, SEF 2		ns	-	< *	-	-	-	> ***	> ***	-	-	-
	SEF 1, SEF 2		> *	-	> ***	-	-	-	ns	ns	-	-	-

NEF		ns	-	ns	-	-	-	> **	< ***	-	-	-
SEF 1	Im, Re	ns	-	ns	-	-	-	ns	> *	-	-	-
SEF 2		ns	-	> *	-	-	-	> *	> ***	-	-	-

Table 1 Continued

	Polychaeta					Amphipods					
	NI 1	NI 2	NI 3	NoF	ShW	NI 1	NI 2	NI 3	NoF	ShW	BOPA-FF
Pair-wise tests for the factors 'BA', 'Lo' and 'Pl'											
Be, Af	-	-	-	> *	-	< *	-	-	-	-	-
NEF, SEF 1	-	< *	-	< ***	-	-	-	-	-	-	-
NEF, SEF 2	-	ns	-	< ***	-	-	-	-	-	-	-
SEF 1, SEF 2	-	> *	-	ns	-	-	-	-	-	-	-
Im, Re	-	-	-	< **	-	-	-	-	-	-	-

Table 2 Results of the 3-factor PERMANOVA for mean sediment grain size, percent organic carbon content and percent organic nitrogen content. Level of significance set at 0.05. Key: Degrees of freedom (df), Mean sediment grain size (MSGS), Percent organic carbon content (POCC), Percent organic nitrogen content (PONC), Not significant (ns), $p < 0.05$ (*), $p < 0.01$ (**), Before (Be), After (Af), Impacted plot (Im), Reference plot (Re), Northeastern farm (NEF), Southeastern Farm 1 (SEF 1), Southeastern Farm 2 (SEF 2)

Source of variation		df	MSGS	POCC	PONC
Before/After = BA		1	ns	**	**
Location = Lo		2	ns	*	ns
Plot = Pl		1	ns	ns	ns
BA x Pl		2	ns	ns	ns
BA x Lo		1	ns	ns	ns
Pl x Lo		2	ns	ns	ns
BA x Lo x Pl		2	ns	*	*
RES		24			
TOT		35			
Pair-wise tests for 3-way interaction term					
NEF	Im	Be, Af	-	< *	ns
	Re		-	ns	ns
SEF 1	Im		-	ns	ns
	Re		-	ns	< *
SEF 2	Im		-	ns	ns
	Re		-	ns	ns
Be	NEF	Im, Re	-	ns	< *
	SEF 1		-	ns	ns
	SEF 2		-	ns	ns
Af	NEF		-	ns	ns
	SEF 1		-	ns	ns
	SEF 2		-	ns	ns

Table 2 Continued

			MSGS	POCC	PONC
Pair-wise tests for 3-way interaction term					
Be	Im	NEF, SEF 1	-	< *	ns
		NEF, SEF 2	-	< **	< *
		SEF 1, SEF 2	-	ns	< *
	Re	NEF, SEF 1	-	ns	> *
		NEF, SEF 2	-	ns	ns
		SEF 1, SEF 2	-	ns	ns
Af	Im	NEF, SEF 1	-	ns	ns
		NEF, SEF 2	-	ns	ns
		SEF 1, SEF 2	-	ns	ns
	Re	NEF, SEF 1	-	> **	< *
		NEF, SEF 2	-	ns	ns
		SEF 1, SEF 2	-	ns	ns

Table 3. BEST results showing the sediment-physico chemical variable, or combination of variables, that best explains the observed variation in the number of individuals of selected indicator taxa, number of families and Shannon-Wiener diversity of polychaetes and amphipods, and the polychaete/amphipod ratio. Level of significance set at 0.05. Key: Before (Be), After (Af), Impacted plot (Im), Reference plot (Re), Northeastern farm (NEF), Southeastern Farm 1 (SEF 1), Southeastern Farm 2 (SEF 2), Number of individuals (NI), Polychaete indicator taxon Maldanidae (1), Paraonidae (2), Glyceridae (3), Amphipod indicator taxon Lysianassidae (1), Urothoidae (2) and Phoxocephalidae (3), Number of families (NoF), Shannon-Wiener diversity (ShW), Mean sediment grain size (MSGS), Percent organic nitrogen content (PONC), Percent organic carbon content (POCC), BOPA-Fish farming index (BOPA-FF), Not significant (ns), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

			NEF		SEF 1		SEF 2	
			Rho	Exp Var	Rho	Exp Var	Rho	Exp Var
Polychaetes	Im	NI 1	-0.055, ns	PONC	0.811, ns	MSGS	0.516, ns	PONC
		NI 2	0.159, ns	POCC	0.370, ns	PONC	0.671, *	PONC
		NI 3	0.290, ns	PONC	0.819, ns	POCC	0.086, ns	POCC, PONC
		NoF	0.525, ns	PONC	0.852, *	POCC	0.215, ns	PONC
		ShW	0.607, *	MSGS, POCC	0.921, ***	POCC, PONC	0.047, ns	PONC
	Re	NI 1	0.488, ns	MSGS, POCC	0.379, ns	POCC, PONC	0.865, *	MSGS, POCC
		NI 2	0.592, ns	MSGS	0.336, ns	POCC, PONC	0.832, *	POCC
		NI 3	0.722, ns	MSGS	0.604, *	MSGS, POCC	-0.145, ns	PONC
		NoF	-0.034, ns	PONC	0.036, ns	MSGS, POCC	0.313, ns	POCC, PONC
		ShW	0.014, ns	POCC	0.157, ns	POCC, PONC	-0.107, ns	MSGS, PONC
Amphipods	Im	NI 1	-0.027, ns	MSGS	0.683, ns	MSGS, POCC	0.013, ns	POCC
		NI 2	0.300, ns	MSGS, POCC	-0.020, ns	PONC	0.461, ns	POCC, PONC
		NI 3	-0.077, ns	POCC	-0.020, ns	PONC	0.470, ns	POCC, PONC

	NoF	0.569, ns	MSGS, POCC	0.400, ns	PONC	0.451, ns	PONC
	ShW	0.530, ns	MSGS, POCC	0.854, ns	POCC, PONC	0.249, ns	PONC
Re	NI 1	0.361, ns	MSGS	0.286, ns	POCC	0.013, ns	MSGS
	NI 2	0.419, ns	PONC	0.379, ns	POCC	-0.134, ns	MSGS
	NI 3	0.283, ns	MSGS	0.123, ns	POCC	0.392, ns	MSGS, POCC

Table 3 Continued

			NEF		SEF 1		SEF 2	
			Rho	Exp Var	Rho	Exp Var	Rho	Exp Var
		NoF	0.073, ns	POCC	0.095, ns	POCC	0.068, ns	MSGS, POCC
		ShW	0.286, ns	PONC	0.146, ns	POCC	0.229, ns	MSGS, POCC
BOPA-FF	Im		0.479, ns	MSGS, POCC	0.743, ns	POCC, PONC	0.410, ns	PONC
	Re		0.125, ns	MSGS	0.754, *	MSGS, POCC	0.525, ns	MSGS, POCC
Polychaetes	Be	NI 1	-0.013, ns	MSGS	0.285, ns	MSGS, POCC	0.669, ns	POCC
		NI 2	-0.120, ns	POCC	0.332, ns	POCC	0.680, ns	POCC, PONC
		NI 3	-0.209, ns	POCC	0.931, **	PONC	-0.083, ns	MSGS, POCC
		NoF	0.361, ns	PONC	0.441, ns	PONC	0.628, ns	MSGS
		ShW	0.136, ns	PONC	0.214, ns	POCC	0.189, ns	MSGS
	Af	NI 1	no test		0.467, ns	PONC	0.495, ns	MSGS, POCC
		NI 2	0.343, ns	MSGS, POCC	0.527, ns	POCC, PONC	0.729, ns	PONC
		NI 3	0.052, ns	MSGS, POCC	0.622, ns	POCC	0.090, ns	MSGS
		NoF	0.521, ns	MSGS, POCC	0.515, ns	POCC, PONC	0.703, ns	POCC, PONC
		ShW	0.668, *	MSGS, POCC	0.780, ns	POCC, PONC	0.206, ns	PONC
Amphipods	Be	NI 1	-0.182, ns	MSGS, PONC	0.381, ns	POCC	0.358, ns	POCC
		NI 2	0.619, ns	MSGS, PONC	0.185, ns	POCC	0.088, ns	POCC
		NI 3	0.683, ns	PONC	0.386, ns	POCC	0.766, *	MSGS
		NoF	0.586, ns	PONC	-0.185, ns	MSGS	0.702, ns	PONC
		ShW	0.568, ns	MSGS	0.143, ns	POCC	0.693, ns	PONC
	Af	NI 1	0.459, ns	MSGS, POCC	0.815, *	POCC	0.130, ns	MSGS
		NI 2	0.030, ns	MSGS, POCC	0.192, ns	MSGS, POCC	-0.056, ns	PONC
		NI 3	0.170, ns	MSGS, POCC	0.274, ns	MSGS	-0.139, ns	POCC
		NoF	0.613, *	MSGS, POCC	0.522, ns	MSGS, POCC	-0.165, ns	MSGS
		ShW	0.810, **	MSGS, POCC	0.871, **	POCC	-0.086, ns	POCC
		Be	0.689, ns	MSGS, PONC	0.579, ns	PONC	0.607, ns	MSGS, POCC
	BOPA-FF	Af	0.404, ns	MSGS, POCC	0.700, ns	MSGS, POCC, PONC	0.114, ns	POCC

Table 3 Continued

			Impacted		Reference	
			Rho	Exp Var	Rho	Exp Var
Polychaetes	Be	NI 1	0.468, ns	MSGS	0.136, ns	PONC
		NI 2	0.256, ns	PONC	0.230, ns	PONC
		NI 3	0.720, **	POCC	0.257, ns	PONC
		NoF	0.511, *	MSGS, POCC, PONC	0.776, **	MSGS, POCC, PONC
		ShW	0.472, *	PONC	0.459, ns	MSGS, PONC
	Af	NI 1	-0.018, ns	MSGS	0.533, ns	POCC
		NI 2	0.03, ns	POCC, PONC	0.200, ns	MSGS
		NI 3	-0.061, ns	MSGS	0.083, ns	MSGS
		NoF	0.241, ns	POCC, PONC	0.295, ns	MSGS, POCC, PONC
		ShW	0.396, *	POCC	0.284, ns	POCC
Amphipods	Be	NI 1	0.199, ns	PONC	0.100, ns	POCC
		NI 2	-0.021, ns	MSGS	0.403, ns	PONC
		NI 3	0.766, **	POCC, PONC	0.55, ns	PONC
		NoF	0.830, **	POCC, PONC	0.349, ns	MSGS
		ShW	0.726, **	PONC	0.252, ns	MSGS
	Af	NI 1	-0.039, ns	POCC	0.128, ns	MSGS, PONC
		NI 2	0.027, ns	MSGS	0.046, ns	MSGS, PONC
		NI 3	-0.014, ns	MSGS	-0.037, ns	PONC
		NoF	0.218, ns	POCC, PONC	0.102, ns	MSGS, POCC, PONC
		ShW	0.382, ns	POCC, PONC	0.096, ns	MSGS, POCC, PONC
	Be	BOPA-FF	0.460, ns	MSGS, POCC, PONC	0.340, ns	MSGS, POCC
	Af		0.329, ns	POCC	0.202, ns	MSGS, PONC