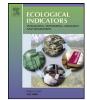
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## Evaluating the influence of off-shore cage aquaculture on the benthic ecosystem in Alghero Bay (Sardinia, Italy) using AMBI and M-AMBI

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#### ABSTRACT

The impact of an off-shore fish farm in Alghero Bay (northwest Sardinia, Italy) on the benthic ecosystem was investigated in 2007 and 2008. In addition to studying the chemical and physical characteristics of the area (i.e., currents and sediment analyses), some biological analyses were also performed. The AZTI's Marine Biotic Index (AMBI) and the multivariate AMBI (M-AMBI) were calculated, which are being used in assessing the ecological status of benthic communities within the European Water Framework Directive (WFD). Clear impact gradients were detected according to both methods; they are related to farm production, prevailing currents, and characteristics of the area (i.e., water depth and distance to the cages). The site affected most was detected within 84 m from the cages; the area that no longer showed effects was over 907 m from the cages. The gradient is shown by decreasing AMBI values and percentage of opportunistic species and increasing richness, diversity, and the presence of sensitive species. This study highlights the importance of setting reference conditions for different areas when calculating M-AMBI. These reference conditions correspond to those in undisturbed sites in the opposite direction of the prevailing currents within the area.

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#### 1. Introduction

Human needs are increasingly affecting marine environments worldwide today (see, e.g., Halpern et al., 2008). One of these effects involves marine aquaculture, which continues to expand globally and which has brought benefits to society, often in fragile coastal communities where traditional employment opportunities are in decline (FAO, 2007). However, many investigations have demonstrated that aquaculture can have a negative impact on the environment (Black, 2001; Buschmann et al., 2006; Giles, 2008; Kalantzi and Karakassis, 2006). In general, these effects can be related to waste products (dissolved and particulate nutrients and organic matter, chemicals, and medicines) from food, fish feces, and excretion. The impact on the benthic ecosystems depends on the amounts released, the time over which they are released, and the assimilation capacity and flushing ability of the local recipient water body (Carroll et al., 2003; Karakassis et al., 2000; Wu, 1995). The aquaculture-environment interaction is well documented (Aguado-Gimènez et al., 2007), the main factors of

this interaction that control the extent of the benthic organic enrichment being farm size, husbandry methods, and hydrographic conditions (Giles, 2008; Hartstein and Rowden, 2004; Mente et al., 2006). Hence, benthic indicators have been used extensively to assess the impact of aquaculture (Buschmann et al., 2006; Gowen and Bradbury, 1987; Gyllenhammar and Hakanson, 2005; Kalantzi and Karakassis, 2006) and, in recent years, several indices have been proposed as ecological indicators in estuarine and coastal waters to assess human-induced impact (see reviews in Díaz et al., 2004; Pinto et al., 2009). Among these indicators, the AZTI's marine biotic index (AMBI) (Borja et al., 2000) has been successfully applied to detect and assess different impact sources worldwide, including aquaculture (Aguado-Gimènez et al., 2007; Bouchet and Sauriau, 2008; Callier et al., 2008; Carvalho et al., 2006; Muxika et al., 2005; Nickell et al., 2009; Sanz-Lázaro and Marín, 2006; Tomassetti et al., 2009). More recently, a new index (M-AMBI or multivariate-AMBI) (Borja et al., 2004; Muxika et al., 2007), which includes AMBI, richness, and Shannon's diversity, has been proposed for assessing the ecological status within the European Water Framework Directive (WFD) (Borja et al., 2004, 2009a). M-AMBI has been also tested with regard to the effects of aquaculture (Bouchet and Sauriau, 2008; Callier et al., 2009; Tomassetti et al., 2009).

In this regard, Borja et al. (2009b) state that the assessment of benthic community response to organic enrichment from aquacul-

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ture may be improved by using a suite of benthic indicators (rather than a single indicator, e.g., M-AMBI instead of AMBI alone), and by considering variables that are unique to the location being studied, e.g., water depth, hydrodynamics, years of farm activity, and total annual production. According to these authors, we can predict benthic impact by taking into account the aforementioned environmental variables using indices such as AMBI. Hence, they recognize that assessments that do not consider these factors could lead to an incorrect interpretation of benthic response. On the other hand, the use of assessment methods developed for the WFD, such as M-AMBI, requires that reference conditions (Muxika et al., 2007) specific for each type or habitat are set, which can represent a limitation when the number of habitats is too high (de Paz et al., 2008; Teixeira et al., 2008).

Hence, the objectives of the present study are: (i) to assess the impact of aquaculture on benthic assemblages, using AMBI and M-AMBI indices (setting reference conditions for the latter); and (ii) to compare observed and predicted AMBI values, taking into account hydrographic and managerial variables for an offshore fish farm in northwest Sardinia (Italy).

#### 2. Materials and methods

#### 2.1. Study area

The study was carried out at a fish farm of 2.5 ha located in the Alghero Bay of the Mediterranean Sea (North Western Sardinia) (Fig. 1a). The sea bottom is flat, with a mean water depth of 38 m. The sampling activities were performed during the month of September for 2 consecutive years, 2007 and 2008, at the end of the biological cycle of the farmed fish. About 116,000 seabass (Dicentrarchus labrax) and 380,000 seabream (Sparus aurata) were being reared in 9 "tension-legs" cages (REFA®). Cage volume was 800 m<sup>3</sup> (5 cages) and 2500 m<sup>3</sup> (4 cages), and the fish density ranged from 0.4 to  $4 \text{ kg m}^{-3}$ . Fish were fed with commercially produced, extruded pellets (Aller Aqua<sup>®</sup>; 42–56% dry matter (d.m.) protein, 18-21% d.m. crude fat, 7.5-12% ash, 0.5-2.5% d.m. crude fiber and 1.1-1.4% d.m. phosphorus) and the daily ratio ranged from 40 to  $190 \text{ kg cage}^{-1}$ , with a total daily average of  $98 \text{ kg cage}^{-1}$ . Total production of the farm was 99t in 2007 and 99.3t in 2008.

#### 2.2. Current meter data

The main surface currents ran parallel to the bay perimeter, moving from SW to NE (APAT, 2008). Nevertheless, the water current speed and the prevailing direction were determined from July to December 2007 at four sampling stations along the vertices of the area granted for farming (Fig. 1b). Currents were measured at three different depths in the water column: 33, 23, and 13 m above the sea bottom, for 15 days at each meter position and for a period of 10 min, using a Sensor Data Current Meter (model SD 2000).

#### 2.3. Sampling design

During the month of September 2007, eight stations were sampled: four stations located close to the cages (stations I, in Fig. 1b) and four stations located at a distance from the cages in the directions of the four cardinal points (stations O, in Fig. 1b). Taking into account the information from the surface current data and the current meter data, a transect of four stations was established along the prevailing direction of the water current at increasing distances from the cages in September 2008 (stations T, in Fig. 1b). In both years, three replicates of sediment samples were collected at each sampling station by divers using "sampling boxes" ( $15 \text{ cm} \times 30 \text{ cm} \times 8 \text{ cm}$ ); the samples were immediately frozen ( $-20 \,^{\circ}\text{C}$ ) and transported in refrigerated containers to the laboratory. Three replicates of the macrofauna samples were collected from each station using a 0.132 m<sup>2</sup> (121 in volume) Van Veen grab sampler; the content of the grabs was sieved with a 0.5mm mesh fixed in 10% buffered formalin and then transferred to the laboratory for further analysis.

#### 2.4. Abiotic parameters

The sediment grain size was analyzed by using a mechanical shaker and dry sieving through a tower of sieves (from 25- to 0.064-mm mesh) and classified according to the Wentworth scale (Buchanan, 1984): 64–2 mm gravel, 2–0.25 mm sand, 0.25-0.065 mm fine sand, and <0.065 mm residual matter (silt and clay). To determine the percentage of water in the sediment (SWC), 500 g of sediment was dried in a stove at 60 °C until the weight was constant and the loss of weight in percentage represented the SWC. The organic matter (OM) was determined as the loss on ignition (LOI) after 5 h at 450 °C in a furnace After that, sediment was burned at 1000 °C to evaluate the carbonate fraction (Dean, 1974; Froelich, 1980). For the sediment sampled in 2008, redox potential (Eh), total nitrogen (TN), total carbon (TC), organic carbon (OC), total sulfur (TS), and total phosphorus (TP) were also determined. Eh was measured on the upper layer sediment, in situ, using an Orion platinum electrode model 9678BNWP (Thermo Scientific<sup>®</sup>). The Carlo Erba Instrument EA1108 Elemental Analyzer (Carlo Erba Inst., Milan, Italy) was employed to determine TN, TC, OC, and TS, whereas the digestion by perchloric acid method was used for TP, according to Olsen and Sommers (1982).

#### 2.5. Biotic parameters

In the laboratory, faunal samples were preserved in 70% ethyl alcohol; organisms were extracted, identified, and counted to the highest possible taxonomic separation, usually species level. These biological quantitative data were used to calculate AMBI and M-AMBI. The observed AMBI values were derived using AMBI Software (version 4.1), which can be downloaded from http://ambi.azti.es. According to Borja et al. (2009b), predicted AMBI values were calculated by using the equation:

#### Predicted AMBI = 4.496 - (0.0486 De) - (1.615C) + (0.000665P)

$$-(0.593 \, \text{Di})$$

where De is depth at each sampling station, expressed as a square root (m); *C* is the current speed, expressed as log (cm  $s^{-1}$ ); *P* is the production, expressed in tons  $yr^{-1}$ ; and Di is the distance of each station to the cages, expressed in log (1+*m*). The predicted values calculated were compared to the observed values to check the fitting of the observed values to the general model.

As M-AMBI requires both bad and high reference conditions (see Muxika et al., 2007) for comparison with monitoring data, five different scenarios of high status were tested, including (i) those from the Italian Adriatic coast (Occhipinti-Ambrogi et al., 2009); (ii) those from a station (O2), in the opposite direction of the prevailing currents; (iii) the lowest AMBI value and highest diversity and richness values from the area; and (iv) two more scenarios, increasing richness and diversity and decreasing AMBI, as a preventive measure, if the area is globally affected by the aquaculture activity. As for bad status, all references were based upon the azoic situation (diversity and richness equal to 0 and AMBI equal to 6).

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