



1	A Generalization of the TRIX trophic index to the Adriatic Sea basin
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24	Abstract
25	The Marine Strategy Framework Directive is pushing for new methodological approaches in
26	order to protect the marine environment more effectively. The trophic index TRIX was
27	developed by Vollenweider in 1998 for the coastal area of Emilia-Romagna (northern
28	Adriatic Sea), and was exploited by Italian legislation to characterize the trophic state of
29	coastal waters. In order to implement TRIX in different areas and for different time periods,
30	we developed a methodology for the generalization of the index changing the scaling
31	parameters.
32	We compared the TRIX index calculated from in situ data ("in situ TRIX") with the
33	corresponding index simulated with a coupled physics and biogeochemical numerical model
34	("model TRIX") implemented in the overall Adriatic Sea. The comparison between in situ
35	and simulated data was carried out for a data time series in the Emilia-Romagna coastal strip.
36	This study demonstrates the compatibility of the model with the in situ TRIX and the
37	necessity to have time series longer than 10 years to evaluate properly the scaling parameters.
38	The model TRIX is finally calculated for the whole Adriatic Sea showing trophic index
39	differences across the Adriatic coastal areas.
40	
41	1. Introduction
42	Marine habitats are subject to increasing pressures (as nutrients discharges, eutrophication)
43	due to agriculture, industry, tourism, fishing, and aquaculture. The eutrophication of coastal
44	waters is considered to be one of the greatest threats to the health of marine ecosystems. It is
45	described as a change in the marine food web connected to the seawater enrichment by
46	nutrients, which can modify the carbon pathways and excessive oxygen consumption
47	(Ferreira et al., 2011; Vollenweider et al., 1992).
48	In response to these pressures, the Marine Strategy Framework Directive (MSFD,

49 2008/56/EC) explicitly considers eutrophication descriptors as key to determining the Good





50	Environmental Status (GES) of European coastal waters. The MSFD underlines the need to				
51	implement an ecosystem-based approach to determine all the pressures affecting the marine				
52	environment relatively to the GES. Indicators therefore need to be developed to qualitatively				
53	and quantitatively assess the quality of the marine environment. Marine ecosystems also				
54	present high levels of complexity; hence indicators are needed to support monitoring				
55	programs and reduce complexity for early warning systems.				
56	Eutrophication assessment indicators should use multivariate water column state variables,				
57	integrating physical-chemical and biological variables. TRIX is an eutrophication index,				
58	proposed by Vollenweider et al. (1998) in order to characterize the trophic state of marine				
59	waters along the Emilia-Romagna coastal region (North Western Adriatic Sea). TRIX is				
60	defined by four state variables, which are strongly correlated with primary production:				
61	chlorophyll-a, oxygen, dissolved inorganic nitrogen and total phosphorous. The TRIX index				
62	was integrated into Italian law in order to monitor the status of the coastal marine				
63	environment (D.L. 260/2010 table 4.3.2/c).				
64	TRIX covers a wide range of trophic conditions from oligotrophy to eutrophy and it has been				
65	applied to coastal marine waters in several European seas: the Adriatic Sea and the				
66	Tyrrhenian Sea (Giovanardi and Vollenweider, 2004), the Black Sea (Kovalova and				
67	Medinets, 2012; Baytut, 2010; Dayatlov et al., 2010; Medinets et al. 2010; Moncheva and				
68	Doncheva 2000; Moncheva et al., 2002; Zaika, 2003), the eastern Mediterranean Sea (Tugrul				
69	et al., 2011), the Aegean Sea (Yucel-Gier et al., 2011), the Marmara Sea (Balkis et al., 2012),				
70	the Caspian Sea (Shahrban and Etemad-Shahidi, 2010), the Mar Menor Lagoon (Salas et al.,				
71	2008), the Persian Gulf (Zoriasatein et al., 2013), and the Gulf of Finland (Vaschetta et al.,				
72	2004).				
73	However, the general methodology for constructing TRIX for the different areas has not been				
74	clarified. In order to apply TRIX to different areas a precise evaluation of the scaling				
75	parameters is required.				





76	In this paper we review the methodology and explain how to adapt TRIX to different coastal
77	and open ocean areas. The specific objectives of our work are: (1) to develop a generic TRIX
78	index equation for the coastal and open ocean areas in the entire Adriatic Sea; (2) to adapt the
79	TRIX to numerical ecosystem model simulation data; (3) to validate the "model TRIX" with
80	in situ data for a long time period. The final results of this paper present a new TRIX index
81	formulation that could be used to assess the GES of coastal and marine waters in terms of
82	eutrophication status.
83	Section 2 describes the TRIX equation and its calibration parameters. Section 3 illustrates the
84	in situ and simulation model data used for the evaluation of TRIX and its calibration. Section
85	4 compares the "in situ TRIX" and "model TRIX", and the sensitivity analysis of the
86	calibration parameters. Section 5 shows how TRIX could be implemented for the whole
87	Adriatic Sea region and Section 6 presents the discussion and conclusions.
88	
89	2. TRIX equation and parameterizations
90	The TRIX index was developed by Vollenweider et al. (1992) using data collected between
91	1982 and 1993 by the "DAPHNE" oceanographic division of the Emilia-Romagna Regional
92	Environmental Protection Agency (hereafter referred as ARPAE-DAPHNE). Since 1971
93	ARPAE-DAPHNE has been carrying out a monitoring program (Regione Emilia-Romagna,
94	1981-2013) covering the whole of the Emilia Romagna coastal region. The location of the
95	sampling stations is reported in Fig. 1.
96	The TRIX index is based on four state variables (n) , which are directly related to
97	productivity: chlorophyll-a (Chl, mg m ⁻³), oxygen as the absolute percentage deviation from
98	oxygen saturation (DO, %), dissolved inorganic nitrogen (DIN, mg m ⁻³) and total
99	phosphorous (<i>TP</i> , mg m ⁻³). In particular, $DIN = NO_3 + NO_2 + NH_4$ and $DO = 100 - Ox $,
100	where Ox is the oxygen saturation. Each state variable is scaled by the highest (U_i) and the
101	lowest (L_i) values in the data time series and TRIX is defined as:

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$$TRIX = \frac{k}{n} \sum_{i=1}^{n} \frac{(\log M_i - \log L_i)}{(\log U_i - \log L_i)}$$
 Eq. 1.1

104 where k=10 is another scaling factor, *n* is the number of state variables considered, and M_i are

the observed *Chl*, *DO*, *DIN* and *TP* values.

106 Vollenweider et al. (1998) further simplified the TRIX formula by assuming (on the basis of

107 the data used) that the difference ($\log U - \log L$) was equal to 3 for all state variables.

108 Therefore, considering k=10, n=4 and the specific log L_i values (see Table III in Vollenweider

to et al., 1998), the TRIX formula was rewritten as follows:

110
$$TRIX = \frac{10}{12} \left[\left(\log M_{chl} + 0.5 \right) + \left(\log M_{DO} + 1 \right) + \left(\log M_{DIN} - 0.5 \right) + \left(\log M_{TP} + 0.5 \right) \right]$$

111 or:

112
$$TRIX = \frac{\left[\log(Chl \times DO \times DIN \times TP) + 1.5\right]}{1.2}$$
Eq 1.2

113 Equation (1.2) gives the TRIX index currently used by ARPAE-Daphne and adopted by the 114 Italian national legislation (D.L. 260/2010). For the Italian coastal waters, TRIX values range from 0 to 10: 0 corresponds to extreme oligotrophic conditions; while 10 corresponds to 115 116 extreme eutrophic conditions. TRIX values have been further aggregated into four trophic 117 regimes (Rinaldi and Giovanardi, 2011): "Elevated", "Good", "Mediocre" and "Bad" (Table 118 1). Referring to Italian waters, TRIX values exceeding 6 are typical of highly productive 119 coastal areas, characterized by frequent episodes of anoxia at the sea bottom (Giovanardi and 120 Vollenweider, 2004). In the following sections we revise the TRIX index scaling parameters U_i and L_i on the basis 121 122 of different (observed and simulated) data sets, in order to evaluate the possibility of applying

the TRIX to numerical simulation data and extend its calculation to open ocean areas. The

assessment is carried out by closely comparing the simulated TRIX ("model TRIX") with

125 corresponding values from in situ observations ("in situ TRIX").







3. In situ and model data
3.1 In situ data
The in situ data used in this paper were collected by the ARPAE-DAPHNE monitoring
program. We considered the 1982-1993 data time series originally used by Vollenweider et
al. (1998) to calibrate in situ TRIX and an additional recent time series covering the period
2001-2012 to validate the model TRIX. The monitoring grid considers 21 sampling stations
located along 8 transects perpendicular to the coast: 19 stations are coastal, extending from
500 m to 10 km offshore, while 2 stations are at 20 a km distance, sampling an open shelf
regime. All the stations are monitored weekly. ARPAE- DAPHNE divided the monitored
area into three sub-areas (A, B and C in Fig. 1) on the basis of the hydrological and trophic
conditions (Montanari et al., 2006). Area A, is located immediately south of the Po delta and
is directly affected by river runoff and nutrient load (see river Po in Fig.1); it is therefore
characterized by enhanced primary production. Area B is a transition area, while area C is
characterized by hydrographical conditions mainly governed by the large-scale basin
circulation.
The in situ TRIX was calculated for each station (using surface values of <i>Chl</i> , DO% <i>DIN</i> and
TP) and averaged over the transects and the three subareas. At the Porto Garibaldi and
Cesenatico transects (see Fig.1) TRIX was calculated with and without the open shelf

stations.

3.2 Numerical model data

148 The model data used in this study were produced by the three-dimensional coupled

- 149 circulation-biogeochemical model consisting of the Princeton Ocean Model, POM (Blumberg
- and Mellor, 1987) and the Biogeochemical Flux Model-BFM (Vichi et al., 2007). The model





151	was implemented in the Adriatic Sea at a horizontal resolution of about 2 km, and 27 sigma-			
152	layers defined the vertical resolution (Clementi et al., 2010).			
153	BFM is a complex lower trophic marine biogeochemical model. It is a biomass based model,			
154	designed to simulate the main marine biogeochemical fluxes through a description of the			
155	ecological functions of the producers, decomposers and consumers and their specific trophic			
156	interactions in terms of basic elements (carbon, nitrogen, phosphorous, silicon and oxygen)			
157	flows. The biological constituents of the model are organized into Chemical Functional			
158	Families (CFFs) and Living Functional Groups (LFGs). CFFs are divided into organic (living			
159	and non-living) and inorganic compounds, which are measured in equivalents of major			
160	chemical elements or in molecular weight units. BFM receives information from the			
161	hydrodynamic model regarding temperature and salinity in order to calculate oxygen			
162	saturation.			
163	The simulations were carried out for the period 1980-2010. Nutrients, oxygen and chlorophyll			
164	values were extracted at the model grid points nearest to the in situ sampling stations (grey			
165	shaded areas in Fig. 1). The model TRIX was then defined with U_i and L_i values computed			
166	using different time periods: 1982-1993 ("model TRIX"), 1991-2010 ("model TRIX 1"),			
167	2001-2010 ("model TRIX 2") and 2006-2010 ("model TRIX 3"). The values obtained are			
168	reported in Table 2 and the model TRIX estimated from these different scaling parameters			
169	were compared with in situ values.			
170				
171	4. Comparison of TRIX estimates from in-situ and simulated data.			
172	Figure 2 shows the comparison of the in situ and model TRIX considering the upper (U_i) and			
173	lower (L_i) values of each state variable obtained from the observed and simulated 1983-1992			
174	time series respectively.			
175	The figure clearly shows that the model TRIX lies in the same range of the in situ TRIX and			
176	the two time series show a distinct seasonal cycle with some degree of similarity. We			





177	computed the correlation coefficient using the simulated and the observed data series for the
178	three sub-areas of the monitored region. The correlation coefficient values are reported in
179	Table 3 and they show decreasing values from area A to C.
180	Area A has the highest correlation values since it is the most eutrophic area, exhibiting the
181	highest TRIX values (> 6), depending on the direct influence of the fresh water runoff and
182	nutrient load from the Po river delta (Fig. 1) immediately northward of area A. Areas B and
183	C show progressively reduced TRIX values (Fig. 2B and C) denoting more oligotrophic
184	conditions and reduced correlation values. This is mostly due to a model slightly
185	underestimation of the index in area B between 2003 and 2006. Area C shows the lowest
186	correlation because there is a temporal phase shift (of about two months) between the two
187	time series between the 2005-2008. We presume that this is due to the particular Po river
188	runoff and climatic conditions that are not well reproduced by the model.
189	In all the three study areas, TRIX increases between 2008-2010. Area A shows values above
190	6 ("bad" water quality conditions, see Table 1) in late winter-early spring and late summer-
191	early autumn (Fig. 2). Areas B and C are characterized by TRIX values that indicate
192	"Mediocre" (5 < TRIX < 6 in winter-spring), to "Good" (4 < TRIX < 5 in summer-autumn)
193	conditions. However, some high in situ TRIX events (> 6) were recorded also in Area B
194	during spring 2004 and 2010, while the "model TRIX" simulates values below 6 for all the
195	period.
196	The TRIX index calculated as averages of each monitoring transect (Lido Volano,
197	Casalborsetti, Ravenna, Lido Adriano, Rimini, Cattolica, Porto Garibaldi and Cesenatico, in
198	Fig. 1) instead of averages over the areas A, B, C provides indications that are consistent
199	with the previous results (see Fig 3). The correlations values relative to each transect are
200	reported in Table 4 for all the transects and again highlight that the overall qualitative
201	agreement between model and in situ TRIX, is reduced moving along an eutrophy to
202	oligotrophy (north to south) gradient.





203	A preliminary conclusion is that model TRIX agrees, within the two standard deviations
204	range, with the corresponding in situ index. In addition the model TRIX estimates appear
205	more coherent with in situ estimations for trophic conditions characterized by a tendency to
206	eutrophy.
207	
208	4.2 Model TRIX sensitivity analysis
209	A sensitivity analysis was carried out by comparing the TRIX index calculated only with the
210	coastal stations, up to 10 km distance from the coast (Fig. 4a and 4b), and the TRIX index
211	computed also considering the two offshore stations, situated at 20 km off the coast for the
212	Porto Garibaldi and Cesenatico transects (Fig. 4c and 4d). The correlation coefficient
213	decreased for the Porto Garibaldi transect and did not change for the Cesenatico transect
214	(Table 4). This further confirms that TRIX is most useful for coastal areas, under the direct
215	influence of river runoff.
216	Another sensitivity analysis was carried out by calculating the TRIX from simulated data
217	using U_i and L_i scaling parameters from three more recent time series with a different
218	temporal length years: 1991-2010 ("model TRIX 1"), 2001-2010 ("model TRIX 2") and
219	2001-2006 ("model TRIX 3") (see Table 2). The sensitivity of the TRIX to the three different
220	sets of scaling values was estimated by computing the percentage difference (PD) between
221	the model TRIX 1, 2 and 3 and the model TRIX obtained using the 1983-1992 data series:
222	$PD_{i} = \frac{(TRIX_{i} - TRIX_{83-92})}{TRIX_{83-92}} $ 1.3
223	where $i=1,2,3$. The PD values are shown in Fig. 5 and illustrate the role on the TRIX values
224	of the time series extension and specific period chosen for the scaling parameters. A shorter
225	time series (5 and 10 years) results in ~10% differences between estimates. Conversely the

- 226 "model TRIX 1" computed with scaling parameters originating from a 20 years time series
- show values close to the model TRIX values calibrated with in situ data (Fig. 5).





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- The correlation between the in situ TRIX and the TRIX calculated from model simulations
- 229 ("model TRIX" and "model TRIX 1, 2 and 3") varied depending on the time period
- 230 considered (Table 3). Generally, area A was the best-fitted area, with high correlation values
- for all the time periods studied. Correlation values decreased in areas B and C when we
- considered short time period data (5-10 years). However a high correlation was observed, in
- all the three areas, between the "in situ TRIX" and the "model TRIX 1" calculate using U_i
- and L_i values from a long time series (20 years) (Table 3).
- 235 In conclusion this sensitivity study shows that TRIX values become independent on the
- scaling parameter values if the time series is longer than 10 years. In all cases, model values
- are better correlated to in situ ones in eutrophic as compared to quasi-oligotrophic areas and
- in near shore than offshore areas.

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240 5. Adriatic Sea model TRIX

- Finally, the model TRIX was computed for the whole Adriatic and Northern Ionian Sea. The
 monthly means computed for the time period 2001-2010 are shown in Fig. 6.
- 243 There is a sharp contrast between the coastal northwestern shores, the southeastern shelves
- and the open sea areas. The northwestern Adriatic Sea shows the highest TRIX values (> 6),
- 245 corresponding to eutrophic conditions that could be related to possible anoxia/hypoxia
- events. The TRIX values progressively decrease along the western Adriatic coast, with
- values ranging between 5 and 6 on the Emilia-Romagna coast, and values < 5 along the
- 248 Marche coast. The southern section of the western Adriatic presents low TRIX values (< 4),
- at the contrary of the south-eastern shelves where eutrophic conditions are prevalent as found
- in previous studies (Marini et al., 2010). The Northern Ionian Sea is generally oligotrophic,
- with TRIX values < 4.



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254 In this paper we have shown a methodology to generalize the TRIX index to an entire sea basin region and to simulation model data. First the TRIX index equation was re-written with 255 general scaling parameters as in Vollenweider et al. (1992). Each state variable is scaled by 256 257 the highest (U_i) and the lowest (L_i) values in the data time series and in our paper we tested the importance of the length and the specific period used to evaluate these scaling 258 259 parameters. 260 The analysis was based on a comparison of in situ data derived TRIX and the model TRIX in 261 the Emilia- Romagna coastal strip. First of all, the results indicated the generally significant 262 potential skill of the model in replicating the observed index, provided that the TRIX scaling parameters are computed from the simulated data set itself. The comparison also indicated 263 264 that in a statistical sense, the model's replication skill decreases consistently with the trophic characteristics tendency towards oligotrophic conditions. This is indicated by the decreasing 265 266 correlation values from area A to areas B and C. This obviously merits further investigation 267 as it is probably related to the main driver controlling the local environmental conditions 268 (external input vs. circulation and vertical structure dynamics). 269 The sensitivity analysis to the extension and specific time series used to evaluate the scaling 270 parameters indicated that for time series longer than 10 years the results were insensitive to 271 the (U_i) and (L_i) values. This is because a long data series is more likely to encompass a 272 wide range of events and better estimate extremes. 273 When implemented in the whole Adriatic Sea basin scale, the model TRIX produced the 274 sharp transition from the eutrophic oriented conditions of the coastal domain, to the 275 oligotrophic conditions that characterise the pelagic domain. 276 Numerical simulations can therefore represent an important support for monitoring activities, i.e. they will allow to extend the use of TRIX to much larger areas where in situ sampling 277 278 activities are difficult to implement.





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382 383

Fig. 1 Emilia-Romagna coastal and shelf region monitored by ARPAE-DAPHNE: 21 stations,

385 organised along 8 transects (Lido Volano, Porto Garibaldi, Casalborsetti, Ravenna, Lido Adriano,

386 Cesenatico, Rimini and Cattolica) from 500 m to 10 Km distance from the coast. The Porto

387 Garibaldi and Cesenatico transects enclose also 2 stations situated 20 Km offshore. The study area

388 is divided in 3 areas (A, B and C) based on hydrological and trophic conditions. The grey shaded

areas indicate the model grid points from which model data were extracted to carry out the "model

390 TRIX".







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Fig. 2 Comparison between "in situ TRIX" (red line) and "model TRIX" (blue line) averaged over 394 the 3 study areas (A, B and C). "Model TRIX" was calculated using U_i and L_i values extracted from









403 Fig. 3 Comparison between "in situ TRIX" (red line) and "model TRIX" (blue line) averaged over 404 six transects (Lido Volano, Casalborsetti, Ravenna, Lido Adriano, Rimini and Cattolica) comprise 405 in the ARPAE-DAPHNE monitoring sampling program. Red and blue shaded areas correspond to average values ± 2 standard deviations. 406

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415 Fig.4 Comparison between "in situ TRIX" (red line) and "model TRIX" (blue line) averaged over

416 two transects (Porto Garibaldi and Cesenatico), including stations up to 10 km distance from the

417 coast (Coastal, a and b), and up to 20 km distance from the coast (Offshore, c and d). Red and blue

418 shaded areas correspond to average values ± 2 standard deviations.

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- 420
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Fig. 5 Fraction differences between the TRIX calculated from simulated data using U_i and L_i values
from three recent time series: 20 years (1991-2010, "model TRIX 1"), 10 years (2001-2010, "model





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- 426 TRIX 2") and 5 years (1991-1995, "model TRIX 3") data series; and the "model TRIX" calculate
- 427 using U_i and L_i from 1983-1992 data series. Values are averaged over the 3 areas (A, B and C).





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Fig. 6 Monthly averages of the "model TRIX" for the Adriatic and Upper Ionian Sea.





432 Table

Conditions	TRIX units	Trophic state	Water quality conditions
Oligotrophic	< 4	Elevated	 Scarcely productive waters Good water transparency Absence of anomalous water colours Absence of Oxygen undersaturation in the bottom waters
	4 < < 5	Good	- Moderately productive waters - Occasionally water turbidity - Occasionally anomalous water colors - Occasionally bottom waters ipoxia episodes
	5 < < 6	Mediocre	 Very productive waters Low water transparency Frequently anomalous waters colours Ipoxia and occasionally anoxia episodes in the bottom layers Suffering of the benthic communities
Eutrophic	> 6	Bad	 Strongly productive waters High water turbidity Diffuse and persistent anomaly in the water colours Diffuse and persistent ipoxia/anoxia episodes in the bottom waters High mortality rate of benthic organisms Alteration of the benthic communities and strong decrease of the biodiversity

Table 1 Reference values of TRIX thropic index and corresponding water quality and trophic

435 conditions, developed from ARPAE -DAPHNE Emilia-Romagna (Rinaldi and Giovanardi, 2011).





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-		-

Time series	limits	Chl	DIN	РТ	DO
1982-1993	$\log L$	-0.72	1.15	0.07	-4.16
	$\log U$	1.12	3.22	2.77	1.96
	$\log U - \log L$	1.84	2.06	2.70	6.12
1991-2010	$\log L$	-0.99	1.84	-0.20	-5.01
	$\log U$	1.02	3.29	2.54	1.84
	$\log U - \log L$	2.01	1.45	2.74	6.85
2001-2010	$\log L$	-0.99	1.84	-0.20	0.001
	$\log U$	0.79	3.13	2.36	1.84
	$\log U - \log L$	1.79	1.29	2.56	4.82
2006-2010	$\log L$	-0.98	1.84	-0.20	-2.84
	$\log U$	0.79	3.11	2.36	1.84
	$\log U - \log L$	1.77	1.27	2.56	4.68

Table 2 Lower (*L*), Upper (*U*) and difference $(\log U - \log L)$ logarithm value of the 4 state variables 452 considered by the TRIX index. Values are extracted from the simulated data series reported in the

453 1st column.

Area	Ν	1982-1993	1991-2010	2001-2010	2006-2010
А	2089	0.64	0.64	0.63	0.63
В	2104	0.58	0.57	0.53	0.53
С	1048	0.36	0.36	0.31	0.30

458459 Table 3 Correlation values between "in situ TRIX" and the TRIX calculated from model

simulations, using U_i and L_i values from different time series: 1982-1993 ("model TRIX"), 1991-

461 2010 ("model TRIX 1"), 2001-2010 ("model TRIX 2"), 2006-2010 ("model TRIX 3").





Transect	Ν	Coastal	Ν	Offshore
Lido Volano	773	0.63	-	-
Porto Garibaldi	788	0.72	906	0.63
Casalborsetti	528	0.70	-	-
Ravenna	496	0.72	-	-
Lido Adriano	784	0.58	-	-
Cesenatico	824	0.48	923	0.48
Rimini	267	0.36	-	-
Cattolica	781	0.34	-	-

469 470

471 Table 4 Correlation values between the "in situ TRIX" and the "model TRIX" for the 8

472 stransects, considering the coastal stations (up to 10 Km distance from the coast) and 2 offshore

473 stations situated in Porto Garibaldi and Cesenatico transects (at 20 Km distance from the coast).

474 For the "model TRIX", the upper and lower limits were extrapolated from simulations carried

475 out during the time period