## ORIGINAL ARTICLE





## Macrobenthic colonization on the derelict Fisheries Vessel Khronometer, Cristo Rey Submarine Park, Argentina

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#### **Abstract**

We investigated the fouling community on the Fisheries Vessel (F/V) Khronometer during a 3-year period, from its sinking, by means of video transects. The results demonstrate that the shipwreck presented high macroinvertebrate coverage from the first survey (100 days after sinking). The most conspicuous organisms were cnidarians, followed by amphipods and serpulids (tubes). The hydroid Ectopleura crocea was the most abundant colonizer in the early stages; the sea anemone Anthothoe chilensis and the azooxanthellate soft coral Tripalea clavaria were abundant 2 years after the vessel sank. Interestingly, shipwreck coverage and species composition at the end of the sampling period (940 days after sinking) were similar to those of the wood vessel Cristo Rey, which sank 36 years before the Khronometer. Besides, some different taxa from that seen on video transects were identified from samples taken during dives, corresponding to isolated organisms with scarce abundance or cryptic habits. Moreover, four reef fish species were recorded. Shipwrecks represent interesting places for recreational activities such as diving and fishing. This is the only study about the early development of an invertebrate community on artificial reefs in Buenos Aires coast, Argentina. Monitoring natural and artificial substrata could help to understand the ecological patterns occurring around them and provide information to help manage artificial reefs in sustainable ways.

#### KEYWORDS

Argentina, artificial reef, benthos, shipwreck, SW Atlantic

## 1 | INTRODUCTION

Artificial reefs (ARs, hard submersed structures) remodel marine ecosystems by creating new habitats providing substratum for settlement of benthic algae and invertebrates, and refuge and nursery areas for fish. Among the different types of ARs, accidentally or deliberately sunken vessels represent particularly interesting places for SCUBA activities, not only because of the high densities of animals observed but also for the aesthetic qualities offered by the structure itself. Consequently, derelict vessels are intentionally deployed on shallow sandy bottom areas to favor recreational diving. The demand for vessel-reefs will undoubtedly escalate as a result of the growing sport diving industry (Arena, Jordan, & Spieler, 2007; Jimenez, Hadjioannou, Petrou, Andreou, & Georgiou, 2017).

In the temperate waters of the Southwestern Atlantic Ocean, SCUBA diving in derelict vessels is particularly popular along the clear waters of the Patagonian coast (e.g., at Las Grutas, 40°48′S, 65°05′W, Don Felix vessel; and at Puerto Madryn, 42°46′S, 65°02′W, Follias, Albatros and Miralles vessels). Northward, at the Buenos Aires coast (38°07′S, 57°33′W), there are two vessel-reefs of interest for divers: the shipwrecks of the freighter James Clunies (149 m length), accidently sunk in 1949 at 10-m depth on rocky floors; and the wood vessel Cristo Rey (45 m length), intentionally sunk on soft bottoms in 1981, at 22 m depth, to create the Cristo Rey Submarine Park (see Genzano, Giberto, & Bremec, 2011).

Despite the recreational and touristic interest in ARs being a consequence of the marine life associated with them, the biological

knowledge of this biota is still scarce. The only study of ARs in the region is of Genzano et al. (2011), who described the macrofaunal assemblages settled on shipwrecks at 38°S, comparing them with the macroinvertebrate assemblages of the neighboring rocky outcrops. After a long-term period of submersion (50 and 36 years for the James Clunies and the Cristo Rey, respectively), the ARs showed a similar community structure to surrounding natural rocky reefs, in concordance with the hypothesis that the ecological processes of ARs are functionally equivalent to those of natural benthic systems in an area (Perkol-Finkel, Miloh, Zilman, Benayahu, & Benayahu, 2006; Seaman, 2000).

During 2014, the big Fisheries Vessel (F/V) *Khronometer* (98 m length) was sunk in order to extend the Cristo Rey Submarine Park. This unique opportunity allowed us to study the colonization and changes in the AR faunal composition during the first months after the sinking. As soon as a ship is submerged, it provides free space to be colonized by sessile epifauna (biofouling). The study of richness and settlement dynamics of these sessile communities helps to understand the development of epibiotic or fouling communities through time. Knowledge of the dynamics of the species colonization on the wreck provides a helpful tool for the management and future expansion of the submarine park.

The aim of this study was to analyse the early development of the invertebrate community on the AR created by the F/V Khronometer shipwreck. To do this, we compared the species composition and coverage of the benthic macrofauna during the period between 3 month and ~3 years after its sinking. Both the benthic community and fish richness associated with the shipwreck were compared with those recorded at the mature AR Cristo Rey, the closest vessel sunk more than 30 years before the F/V Khronometer.

#### 2 | MATERIAL AND METHODS

## 2.1 | Study site

The sublittoral area of Mar del Plata is characterized by sandy sea floors and isolated hard bottoms. The latter are composed of *loess* (consolidated sedimentary rocks, Pleistocene) and quartzite rocky outcrops, which originated from the Tandilia mountainous system (SE of Buenos Aires Province) 550 mya (Genzano, Zamponi, Excoffon, & Acuña, 2002; 2011).

The outcrops and the surrounding areas are subjected to many human activities, including commercial and recreational fisheries and diving. The whole area is affected by a strong northwards littoral current, semi-diurnal tidal currents and high-energy waves (Lanfredi, Pousa, & Dragani, 1992). Storms and winds notably produce sediment re-suspension. Consequently, diving is usually undertaken under dangerous conditions, with water visibility ranging between 0.5 and 6 m depth, with an average of 3 m depth. Water temperature is variable through the year. The maximum values in summer reach 18–19°C and 7–8°C is the minimum in winter (Genzano et al., 2002; 2011).

Shipwrecks have formed ARs during recent decades. Some of these ARs are the products of accidental sinking (e.g., *James Clunies* and *Lady Lewis* vessels), while others were intentionally deployed on the sea floor, e.g., the derelict vessel *Cristo Rey*, which forms the basis of the unique submarine park in the area (for more details see Genzano, 2017 and Genzano et al., 2011). Unlike the other abovementioned shipwrecks, the *Cristo Rey* wreck provides hard substrata suitable for the recruitment of benthic macrofauna and as refuges for fish species.

Diving centers of the region proposed to extend the submarine park to boost diving activities. Consequently, in May 2014, the Russian fishing vessel *Khronometer* (98 m length) was deliberately sunk at 38°07′S, 57°31′W (Figure 1), after the elimination of toxic substances and biofouling, as recommended by the official rules. The vessel wreck lies at 24 m depth on the sandy sea floor; the deck rises up to 16 m depth from the sea floor.

## 2.2 | Benthic species and live cover

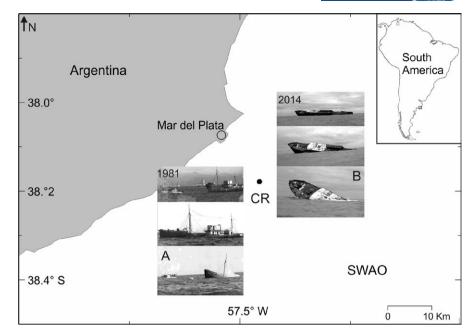
Macrobenthic species and cover (estimated abundance) were determined with the use of video transects obtained through SCUBA diving in August 2014 (100 days after the sinking), November 2014 (175 days), August 2016 (755 days), February 2017 (905 days) and March 2017 (940 days).

Quantitative monitoring of organisms was performed through permanent video transects filmed at 0.45 m above the substrata on the starboard side (at 18 m depth, vertical substrate) and the deck (at 16 m depth, horizontal substrate). The length of transect belts was determined by the extension of benthic assemblages on each substrate and varied from 3 to 8 m in length × 0.25 m wide. The percentage of coverage of species was estimated using a discrete point sampling method described by Osborne and Oxley (1997). Briefly, the recorded images were analysed by playing back the video and viewing on a screen with five points arranged. The video tapes were stopped at set intervals. To provide good estimates of total cover 70-100 points were counted in each transect. Any organism lying beneath each of the five points was identified and classified to the lowest possible taxonomic level. For each organism, percentage cover was estimated by dividing the number of points at which that taxon was recorded by the total number of data points, and multiplying by 100.

Additionally, photos and in situ observations were used in order to identify other attached or motile species that could not be recorded in the video transects. Furthermore, specimens and/or colonies of some organisms were sampled for detailed identification in the lab. Considering the difficult and dangerous diving conditions in the studied area (strong currents and low visibility), underwater video is an accurate option to properly study the benthic community in the area, allowing fast examination of the shipwreck's biodiversity.

## 2.3 | Data analysis

A correspondence analysis (CA) was carried out to explore organisms' cover patterns. A Spearman's rank correlation analysis was also



**FIGURE 1** Location of the Cristo Rey Submarine Park (CR). Sequenced images of the sinking of the *Cristo Rey* (a) and *Khronometer* (b) ships. SWAO. South West Atlantic Ocean

conducted between scores of the two first axes with each taxa's cover.

Finally, a one-way analysis of variance (ANOVA) was performed to study the effect of time since sinking (independent variable) on species cover and the most conspicuous species cover (dependent variables) in horizontal (*n* = 35 per fouling species) and vertical (*n* = 18 per fouling species) substrata. For horizontal substrata, species cover was also compared with the species cover of the *Cristo Rey*, which has a horizontal position. The ANOVA was conducted by constructing a generalized linear model (GLM), which allows modeling response variables with a distribution different from Gaussian. As organism's cover data are proportions, these models have a binomial distribution and a canonical link function was specified (Zuur, leno, Walter, Saveliev, & Smith, 2009). Models were fitted by maximum likelihood estimation.

Statistical analyses were performed using the open access software R (R Core Team, 2016). The CAs were conducted using the capackage (Nenadic & Greenacre, 2007). The ANOVAs of the GLM models and *post hoc* comparisons were performed with the car (Fox & Weisberg, 2011) and Ismeans (Lenth, 2016) packages.

## 2.4 | Fish census

Visual censuses of fish were also conducted in order to compare the richness found on the *Khronometer* with that found on the other neighboring shipwrecks. Censuses were performed by free swimming along the diving site for 15 min per survey and recording all species that could be positively identified (Lessios, 1996). As local species do not form dense fish schools, the species were assigned into one of four relative abundance categories: (i) occasional, a single individual, (iii) scarce, two to five individuals, (iii)

abundant, six to 10 individuals, and (iv) very abundant, more than 10 individuals observed during the survey (viz., Genzano et al., 2011).

#### 3 | RESULTS

# 3.1 | General description of the communities and the benthic cover

Free substrate devoid of fouling organisms ranged between 9% and 34% and 9% and 44% for horizontal and vertical substrata, respectively. The total living cover was high from the first survey (i.e., 100 days after the sinking) in both substrata. However, some significant differences were observed between the sampling periods in both substrata (Figure 2).

Differences in the horizontal total living cover were found with respect to the time since the sinking (Tables 2 and 3; Figure 2). At 175, 905 and 940 days after the sinking, cover values were higher than for earlier surveys, and the values for these later time points were similar. Interestingly, at 940 days, the live cover was similar to the cover of the *Cristo Rey* wreck (Table 3; Figure 2). The main difference recorded between the *Cristo Rey* and the last sampling of the *Khronometer* was regarding the species *Amphisbetia operculata* and *Tripalea clavaria*.

In terms of the cover of the most conspicuous species, *Ectopleura crocea* had the highest values at 100 days; after that, the values decreased and remained relatively constant during the sampling period. By contrast, the pattern of cover of tubes of the Serpulidae *Hydroides plateni* varied over time; however, a slight increase was observed in the later part of the survey (Tables 2 and 3; Figure 3). Gammaridae tubes had a low level of cover after

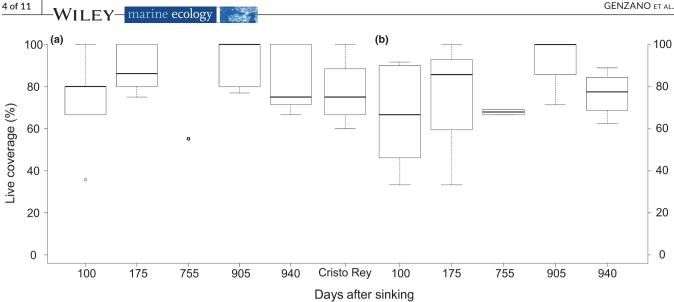


FIGURE 2 Total live cover at different points in time (in days after sinking) on (a) horizontal and (b) vertical surfaces of the F/V Khronometer

100 days of submersion, increasing up to 80%-100% at 175 days, and then it remains at 20% at 755, 905 and 940 days (Tables 2 and 3; Figure 3). In addition, the cover values of E. crocea, serpulids and gammarids on the Khronometer at the end of the sampling period (940 days after the sinking) and those on the Cristo Rey were similar (Tables 2 and 3; Figure 3). Considering the horizontal coverage CAs, the first two axes explained 79.3% of the variance (Figure 4a). The variation explained by the first CA axis is positively correlated with Anthothoe chilensis cover and negatively correlated with that of E. crocea (Table 1). The second CA axis is significantly negatively correlated with cover of A operculata,

Austrobalanus improvisus and Mytilus edulis. The remaining species have no significant correlation with either axis 1 or axis 2 (Table 1; Figure 4).

In terms of the vertical living coverage, there was no variation in total live cover over the study period (Tables 2 and 3, Figure 2). Nevertheless, during the samplings conducted after 100 and 175 days of submersion, the total live cover had a high level of variance, ranging from 30% to 100%. At 905 and 940 days, however, the total cover had a lower range of variation (from 68% to 100%, Figure 2).

Considering the cover of the most conspicuous species over the vertical surface, E. crocea had the highest cover values throughout

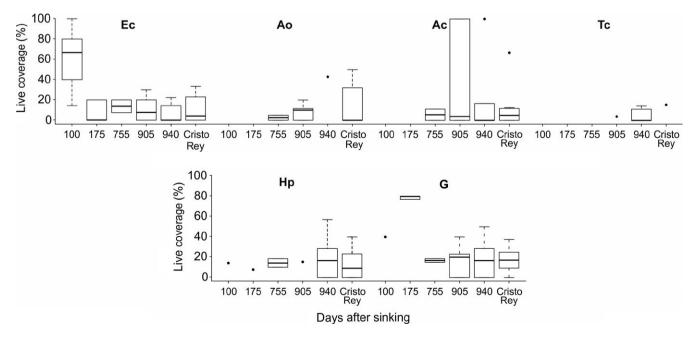
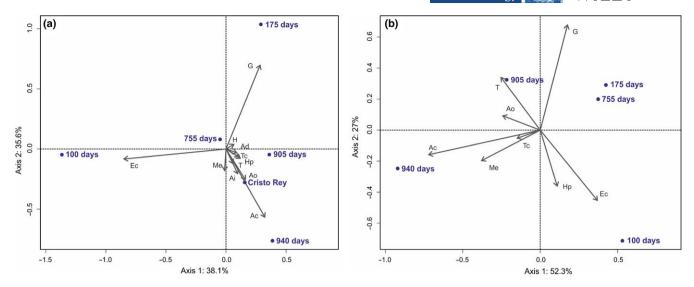


FIGURE 3 Conspicuous organisms' live cover at different points in time (in days after sinking) on horizontal surfaces of the F/V Khronometer. Abbreviations: Ec, Ectopleura crocea; Ao, Amphisbetia operculata; Ac, Anthothoe chilensis; Tc, Tripalea clavaria; Hp, Hydroides plateni; G, Gammaridae



**FIGURE 4** Correspondence analysis scatterplot of the cover data for (a) horizontal and (b) vertical surfaces of the F/V Khronometer. Values labeled on the plot are the numbers of days after sinking. Abbreviations: H, Haplosclerida; Ec, *Ectopleura crocea*; Ao, *Amphisbetia operculata*; Ac, *Anthothoe chilensis*; Tc, *Tripalea clavaria*; Hp, *Hydroides plateni*; G, Gammaridae; Ai, *Austrobalanus improvisus*; Me, *Mytilus edulis*; Ad, *Arbacia dufresnii*, T, Tunicata

TABLE 1 Rho' Spearman correlation values between organisms cover with scores on the two first axes of the correspondence analysis

		Rho' Spearman correlation values		
	Таха	axis 1	axis 2	
Horizontal substrate	Haplosclerida	0.09	0.55	
	Ectopleura crocea	-0.77	-0.23	
	Amphisbetia operculata	0.46	-0.78	
	Anthothoe chilensis	0.64	-0.84	
	Tripalea clavaria	0.58	-0.59	
	Hydroides plateni	0.14	-0.20	
	Gammaridae (tubes)	0.37	0.49	
	Austrobalanus improvisus	0.66	-0.66	
	Mytilus edulis	0.09	-0.80	
	Arbacia dufresnii	0.27	-0.26	
	Tunicata	0.51	-0.86	
Vertical substrate	Ectopleura crocea	0.70	0.00	
	Amphisbetia operculata	-0.78	0.45	
	Anthothoe chilensis	-0.89	0.11	
	Tripalea clavaria	-0.67	-0.34	
	Hydroides plateni	0.41	-0.87	
	Gammaridae (tubes)	0.00	0.70	
	Mytilus edulis	-0.71	-0.35	
	Tunicata	-0.78	0.45	

Significant values are in bold

the sampling period, but decreased considerably at 940 days after the sinking. As for the horizontal substrata, serpulids had an a variable pattern of cover over time. The gammarids had low cover values at 100 days after sinking, a considerable increase up to 80%–100% after 175 days, stabilization of the values at 755 and 905 days, and

a final decrease at 940 days (Tables 2 and 3; Figure 5). Furthermore, the first two CA axes explained 73.7% of the variance (Figure 4b). Variation associated with the first CA axis is positively correlated with *E. crocea* cover and negatively correlated with *A. operculata*, *A. chilensis*, *M. edulis* and solitary tunicate cover (Table 1). The second

**TABLE 2** Analysis of the effects of sinking time (independent variable) on species cover and most conspicuous species cover (dependent variables) in horizontal and vertical substrata

	Horizon	Horizontal substrata			Vertical substrata		
Dependent variables	df	Residual deviance	p-value	df	Residual deviance	p-value	
Live cover	29	42.60	.002	13	23.52	.223	
Ectopleura crocea	29	62.07	<.001	13	21.06	.009	
Hydroides plateni	29	41.43	.052	13	19.29	.899	
Gammaridae	29	38.94	<.001	13	12.37	.001	

Significant p-values (p < .05) are in bold.

CA axis presents no significant correlation with cover of any species (Table 1; Figure 4). Similarly to the horizontal substrata, the first stage of colonization is dominated by *E. crocea*. However, the later stages of colonization are associated with high cover values of *A. operculata*, *A. chilensis*, *M. edulis*, tunicates and low cover of *E. crocea*.

## 3.2 Other benthic organisms on the wrecks

Some organisms that were rarely observed were the red algae Rhodymenia sp., small colonies of Plumularia setacea (Hydrozoa) and Bicelariella sp. (Bryozoa), the bivalves Aequipecten tehuelchus and Ostrea puelchana, the nudibranch Pleurobranchaea maculata, the crabs Platyxanthus crenulatus and Leucippa pentogona, and colonial tunicates. The other species recorded were small-sized cryptic organisms, usually epizoites on big clumps of the colonial hydroids Amphisbetia operculata and Ectopleura crocea, such as the red algae Pterosiphonia sp., Clytia gracilis (Hydrozoa), Bugula sp. (Bryozoa), the microcrustacean Caprella equilibra, the crab Pilumnus reticulatus, the picnogonid Achelia assimilis and Olividae (Gastropoda).

#### 3.3 | Reef fish around the shipwrecks

Four reef fish species were recorded during the five visual censuses conducted during this study. They were all classified as very abundant species. The small Serranidae *Dules auriga* was an early inhabitant of the wrecks, being recorded from the first sampling onwards. In the later part of the study period the Brazilian sandperch *Pinguipes brasilianus* was also very frequent. At the end of the analysed period, *i.e.* nearly 3 years after the sinking, numerous sea bass *Acanthistius brasilianus* (mainly in holes on the deck) and schools of the white beam *Diplodus argenteus* (swimming very close to the wrecks) were observed.

#### 4 | DISCUSSION

Knowledge of the fouling biodiversity over shipwrecks on the Argentinean continental shelf (Southwestern Atlantic) is scarce. Only the shipwrecks of the freighter *James Clunie* and the wood vessel *Cristo Rey* (Genzano et al., 2011) in temperate warm waters of Buenos Aires and the sloop of war *HMS Swift* in temperate cold

waters of Patagonia (Bastida, Grosso, & Elkin, 2008; Elkin et al., 2007) have been studied, decades after they sank. In the present paper, the early colonization of macrobenthos on a shipwreck was analysed.

Video-visual estimation methods have been used widely to monitor species composition on both natural and artificial reefs as they minimize the time spent sampling underwater and reduce costs. Their main limitation lies in the low taxonomic resolution that can be achieved (see Ninio, Delean, Osborne, & Sweatman, 2003); however, the use of videos was considered to be appropriate in the present study area due to the good knowledge of the fauna composition gained from diving experience during recent decades (Excoffon, Acuña, Zamponi, & Genzano, 2004; Genzano, 2017; Genzano et al., 2002; 2011; Meretta & Genzano, 2015). Moreover, some organisms were collected and identified in the lab.

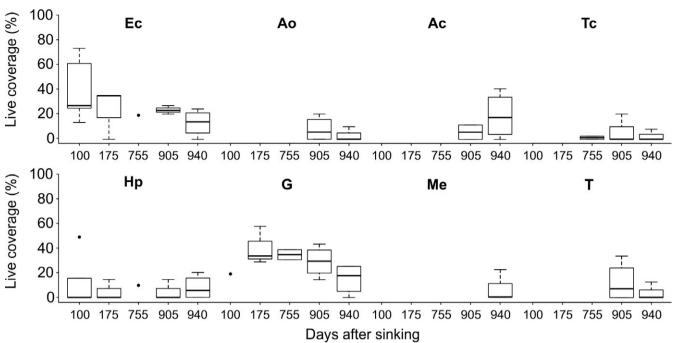
Directly after sinking, shipwrecks are new substrates, quickly colonized by a diverse assemblage of microbial organisms (i.e., biofilms) that change the biological, chemical and physical properties of the substrate. They often act as cues used by invertebrate larvae to locate or identify potential settlement sites allowing the development of abundant and diverse benthic communities (Lozano-Cortes & Zapata, 2014). This well-known process explains the significant amount of living cover recorded during the first months after the sinking of the *Khronometer*: 65%–70% living cover at 100 days. This total coverage of marine epibiota was strikingly high and the same conspicuous species were recorded on both horizontal and vertical substrata.

Remarkably, after nearly 3 years of submersion, the species composition and coverage on the *Khronometer* were similar to those observed on the *Cristo Rey* shipwreck, which sank 36 years before the *Khronometer*. Although there is no biodiversity information over time of any other shipwreck in the area, this similarity may reflect the speed at which invertebrates colonize new bare substrates. The richness and coverage level of the benthic community found on the *Khronometer* are also similar to those of other neighboring natural and artificial hard bottoms (Genzano et al., 2011). These hard bottoms present a horizontal orientation at similar depths; thus, it is not surprising that community structures on artificial and natural hard substrates with similar structural features will become almost indistinguishable (Perkol-Finkel et al., 2006). Perkol-Frinkel et al. (2005) found that, in tropical waters, the species composition found on

**TABLE 3** Multiple comparisons across the numbers of days since the sinking of the F/V Khronometer

		Horizontal substrata			Vertical substrata		
	Comparisons	Estimated (±SE)	z-ratio	p-value	Estimated (±SE)	z-ratio	p-value
Live cover	100-175	-1.41 (±0.58)	-2.41	.154	_	_	_
	100-755	0.33 (±0.45)	0.73	.978	_	_	_
	100-905	-1.14 (±0.51)	-2.24	.222	_	_	_
	100-940	-0.91 (±0.47)	-1.92	.387	-	-	-
	100–Cristo Rey	-0.59 (±0.42)	-1.40	.728	_	-	_
	175-755	1.73 (±0.56)	3.09	.025	-	-	-
	175-905	0.26 (±0.61)	0.43	.998	_	_	_
	175-940	0.49 (±0.58)	0.85	.959	-	-	-
	175–Cristo Rey	0.81 (±0.54)	1.50	.664	_	_	_
	755-905	-1.47 (±0.48)	-3.03	.029	_	-	-
	755-940	-1.24 (±0.45)	-2.78	.060	_	_	_
	755–Cristo Rey	-0.92 (±0.39)	-2.35	.173	_	-	-
	905-940	0.23 (±0.51)	0.45	.998	_	_	_
	905–Cristo Rey	0.55 (±0.46)	1.19	.843	_	-	-
	940–Cristo Rey	0.32 (±0.42)	0.76	.974	_	_	_
Ectopleura crocea	100-175	2.51 (±0.68)	3.68	.003	0.79 (±0.59)	1.33	.669
	100-755	1.92 (±0.54)	3.53	.006	1.34 (±0.46)	2.92	.029
	100-905	2.01 (±0.54)	3.72	.003	1.10 (±0.57)	1.93	.303
	100-940	2.60 (±0.61)	4.26	<.001	1.61 (±0.55)	2.95	.027
	100–Cristo Rey	1.86 (±0.46)	4.07	<.001	_	_	_
	175-755	-0.59 (±0.74)	-0.80	.968	0.55 (±0.61)	0.90	.8988
	175-905	-0.50 (±0.74)	-0.67	.985	0.31 (±0.70)	0.44	.992
	175-940	0.09 (±0.79)	0.11	1	0.82 (±0.68)	1.21	.749
	175–Cristo Rey	-0.65 (±0.68)	-0.95	.934	-	_	_
	755-905	0.09 (±0.62)	0.15	1	-0.24 (±0.59)	-0.41	.994
	755-940	0.68 (±0.68)	1.00	.917	0.27 (±0.57)	0.47	.989
	755–Cristo Rey	-0.06 (±0.54)	-0.11	1	_	_	_
	905-940	0.59 (±0.68)	0.87	.954	0.51 (±0.66)	0.77	.939
	905–Cristo Rey	-0.15 (±0.54)	-0.28	.999	_	_	_
	940-Cristo Rey	-0.74 (±0.61)	-1.21	.834	_	_	_
Gammaridae	100-175	-3.99 (±0.81)	-4.91	<.001	-2.69 (±0.88)	-3.07	.018
	100-755	-1.31 (±0.82)	-1.59	.608	-2.32 (±0.79)	-2.94	.027
	100-905	-1.60 (±0.80)	-1.99	.346	-2.13 (±0.87)	-2.45	.103
	100-940	-1.44 (±0.80)	-1.80	.467	-1.13 (±0.90)	-1.26	.716
	100-Cristo Rey	-1.31 (±0.78)	-1.67	.551	_	_	_
	175-755	2.68 (±0.53)	5.03	<.001	0.37 (±0.58)	0.63	.970
	175-905	2.39 (±0.50)	4.79	<.001	0.56 (±0.69)	0.81	.927
	175-940	2.55 (±0.50)	5.15	<.001	1.55 (±0.73)	2.13	.206
	175–Cristo Rey	2.68 (±0.47)	5.72	<.001	_	_	_
	755-905	-0.29 (±0.52)	-0.57	.993	0.19 (±0.58)	0.33	.997
	755-940	-0.13 (±0.51)	-0.26	.999	1.18 (±0.62)	1.91	.314
	755–Cristo Rey	-0.004 (±0.49)	-0.01	1		_	_
	905-940	0.16 (±0.48)	0.34	.999	0.99 (±0.72)	1.38	.643
	905–Cristo Rey	0.29 (±0.45)	0.64	.988	_	-	_
	940-Cristo Rey	0.13 (±0.45)	0.29	.999	_	_	_

Significant p-values (p < .05) are in bold.



**FIGURE 5** Conspicuous organisms' live cover at different points in time (in days after sinking) on vertical surfaces of the F/V Khronometer. Abbreviations: Ec, *Ectopleura crocea*; Ao, *Amphisbetia operculata*; Ac, *Anthothoe chilensis*; Tc, *Tripalea clavaria*; Hp, *Hydroides plateni*; G, Gammaridae; Me, *Mytilus edulis*; T, Tunicata

shipwrecks are similar to that on natural reefs after approximately 20 years. However, in temperate seas these similarities between natural and artificial substrates can be evidenced very quickly, at ~5 years.

Hard substrata in the marine environment are bi-dimensional structures, but a third dimension generally develops through the growth of sessile species, which act as substrates for others (Fraschetti, Giangrande, Terlizzi, & Boero, 2003). These structural aspects are important factors not only in influencing the settlement and/or survivorship of invertebrates (Zintzen, Norro, Massin, & Mallefet, 2008) but also in providing refuge and food to reef fishes. The fishes observed during the present study are also commonly found in neighboring natural reefs (Genzano et al., 2011). The abundance of the sea bass *Acanthistius brasilianus* is particularly interesting due to its commercial value, reinforcing the role of ARs in the development of artisanal fisheries in the area.

Regarding the development of the invertebrate community, the early colonizers were mainly hydroids and after them, serpulids. During the second year (755 days after sinking) the shipwreck was strongly dominated by cnidarians in terms of abundance (22% coverage) and specific richness, followed by amphipod and serpulid tubes (17% and 14%, respectively). During the last months of the period studied (940 days), cnidarians were the best-represented group in terms of coverage. Similar results have also been observed for other shipwrecks in cold temperate Patagonian areas, where different species of sea anemone also showed dominance in terms of coverage (G. Genzano, personal observations).

Hydroids are pioneer species, able to settle on newly available substrata. The tubularid *Ectopleura crocea* in particular, is a typical fouling species commonly colonizing submerged artificial substrates,

such as shipwrecks (Genzano et al., 2011; Zintzen et al., 2008). The actinula larvae presumably prefer to settle on these artificial substrates rather than the parental hydrocauli because of a more attractive microbial biofilm on the wreck's surface (Di Camilo et al., 2013). In neighboring rocky inter-tidal areas, large colonies composed of numerous unbranched hydrocauli provide a suitable substrate for many associated species (Genzano, 2001). In sublittoral outcrops from the area, clumps of the sertularid *Amphisbetia operculata* are substrata and refuges for many epizoites (Meretta & Genzano, 2015). Both of these hydroids were abundant in the analysed shipwreck showing similar inter-specific relationships, particularly with caprellids, pycnogonids and the syllid parasite *Procerastea halleziana* Malaquin, 1893 (Genzano & San Martín, 2002).

Other cnidarians, the sea anemone A. chilensis and the azooxanthellate soft coral Tripalea clavaria, were also conspicuous 2 years after sinking. As for other ARs in the area (Genzano et al., 2011) the sea anemone A. chilensis formed dense patches of up to 3,000 individuals·m<sup>-2</sup> (G. Genzano, personal observations). Such high abundances of this small sea anemone could be related to its strategy of reproduction by longitudinal fission (Excoffon & Acuña, 1998). This species also occurs in dense patches in the inter-tidal fringe (Excoffon, Belem, Zamponi, & Schlenz, 1997). Another fast-growing sea anemone, Metridium senile, has a similar asexual reproductive mechanism and is often found colonizing shipwrecks in coastal temperate waters in Belgium (Zintzen et al., 2008) and Patagonia (G. Genzano, personal observations). In general, cnidarians are passive suspensivores, which explains their preference to colonize substrata where water currents guarantee a continuous supply of food and avoid the negative effects of sedimentation. Levels of suspended

particles are highly dependent on the existence of elevated structures mostly because the water current velocity increases when passing by the edge of them (Falcâo, Santos, Drago, Serpa, & Monteiro, 2009). Patches of *A. chilensis* and colonies of *T. clavaria*, *A. operculata* and *E. crocea* depend on the flow current to feed both zooplankters and resuspended benthic prey, such as diatoms, caprellids and gammarids (Acuña, Excoffon, & Genzano, 2001; Acuña, Excoffon, Zamponi, Excoffon, Zamponi, & Genzano, 2004; Genzano, 2005).

Only two tube-building organisms were recorded, one serpulid and one gammarid. Serpulids are nearly always associated with hard substrates, usually rocks (Rouse & Pleijel, 2001); although the calcareous tubes provide a hard settlement substrate hard settlement substrates, no epibionts were observed on them.

Amphipods are known as important fouling organisms over natural and artificial hard substrata. They are commonly found as epizoites on colonies of hydroids, in particular *E. crocea* and *A. operculata* (Genzano, 2002; Meretta & Genzano, 2015; Zintzen, Massin, Norro, & Mallefet, , ). These organisms construct tubes to inhabit to prevent them from being washed away (Ulrich, Anger, & Schöttler, 1995) by the strong marine currents characterizing this area. In this study, gammarid coverage concurs with a decrease in that of its conspicuous basibiont *E. crocea* for both vertical and horizontal surfaces.

Although big thalli of algae are not commonly found on the outcrops off Mar del Plata, some encrusting and small macro-algae can be found (Kühnemann, 1972; Negri, Benavides, & Akselman, 2004; Parma, Pascual, & Sar, 1987). Our video transects did not allow us to perform a rigorous census of algae due to their difficulty in detection, their scarce abundance (e.g., Rhodymenia sp.) and their cryptic habits (e.g., Pterosophonia sp.). The alien kelp Undaria pinnatifida, which has an affinity for sheltered areas (Silva, Woodfield, Cohen, Harris, & Goddard, 2002), was recorded at Mar del Plata Port in 2011 (Meretta, Matula, & Casas, 2012). Although the water temperature does not limit further dispersal of U. pinnatifida, the turbidity and high energy conditions along the sampling area are factors that may explain its absence outside Mar del Plata Port. Another invasive species, the nudibranch P. maculata, was recorded at Mar del Plata Port in 2009 and is still found in that area (Farias, Wood, Obenat, & Schwindt, 2016). The species was first found outside of this port on our sampled shipwrecks and on the neighboring outcrops (G. Genzano, personal observations). This sea slug is a voracious predator (Willan, 1984) that feeds on the anemone Anthothoe albocincta (Ottaway, 1977); a potential prey on the studied shipwrecks could be the anemone A. chilensis. Competitive interactions with local predators are expected, which may pose a great impact to the benthic community analysed here. It is worth mentioning that P. maculata specimens from Mar del Plata were positive for neurotoxins (Farias, Obenat, & Goya, 2015), which increases the importance of studying how this species interacts with the different components of the local community.

The sublittoral off Mar del Plata is characterized by the presence of hard outcrops (quartzite rocks) of variable size, surrounded by sand and shell debris and with different physiognomies. These

natural reefs constitute the largest hard substrate in the area (Genzano, 2017; Genzano et al., 2011). Shipwrecks increase the hard substrate available for settlement, reproduction and dispersion of organisms. In this sense, as discussed by Zintzen et al., (2006), shipwrecks could also favor the spread of exotic species, having an undesirable impact on local communities. As ARs occupy a proportion of soft bottoms, it is important that we improve our understanding of impacts on the sedimentary ecosystem structure and function. This is the only study about early development of invertebrate communities on ARs in the area. Monitoring and comparisons between natural and artificial substrata could help to understand the ecological patterns of substrate colonization in order to provide tools to manage ARs in sustainable ways (Dafforn et al., 2015; Heery et al., 2017).

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#### **REFERENCES**

- Acuña, F. H., Excoffon, A. C., & Genzano, G. N. (2001). Feeding of Anthothoe chilensis (Lesson, 1830) (Actiniaria) in Mar del Plata port (Buenos Aires, Argentina). Biociencias, 9, 111–120.
- Acuña, F. H., Excoffon, A. C., Zamponi, M. O., & Genzano, G. N. (2004). Feeding habits of the temperate octocoral *Tripalea clavaria* (Studer, 1878) (Octocorallia, Gorgonaria, Anthothelidae), from sublittoral outcrops off Mar del Plata, Argentina. *Belgian Journal of Zoology*, 134, 65-66.
- Arena, P. T., Jordan, L. K. B., & Spieler, R. E. (2007). Fish assemblages on sunken vessels and natural reefs in southeast Florida, USA. *Hydrobiologia*, 580, 157–171. https://doi.org/10.1007/s10750-006-0456-x
- Bastida, R., Grosso, M., & Elkin, D. (2008). The role of benthic communities and environmental agents in the formation of underwater archaeological sites. In M. E. Leshikar-Denton, & P. Luna Erreguerena (Eds.), *Underwater and maritime archaeology in latin America and the Caribbean* (pp. 173–185). Walnut Creek, CA: Left Coast Press.
- Dafforn, K. A., Glasby, T. M., Airoldi, L., Rivero, N. K., Mayer-Pinto, M., & Johnston, E. L. (2015). Marine urbanization: An ecological framework for designing multifunctional artificial structures. Frontiers in Ecology and the Environment, 13, 82-90. https://doi.org/10.1890/140050
- Di Camilo, C. G., Giordano, G., Bo, M., Betti, F., Mori, M., Puce, S., & Bavestrello, G. (2013). Seasonal patterns in the abundance of *Ectopleura crocea* (Cnidaria: Hydrozoa) on a shipwreck in the Northern Adriatic. *Marine Ecology*, 34, 25–32.
- Elkin, D., Argüeso, A., Bastida, R., Dellino-Musgrave, V., Grosso, M., Murray, C., & Vainstub, D. (2007). Archaeological research on HMS Swift: A British Sloop-of-War lost off Patagonia, Southern Argentina, in 1770. International Journal of Nautical Archaeology, 36, 32–58. https://doi.org/10.1111/j.1095-9270.2006.00117.x
- Excoffon, A. E., & Acuña, F. H. (1998). Dinámica reproductiva y aspectos poblacionales de un clon de Anthothoe chilensis (Lesson, 1830) (Cnidaria, Anthozoa) del intermareal de Mar del Plata. Frente Marítimo, 17, 107-111.
- Excoffon, A. C., Acuña, F. H., Zamponi, M. O., & Genzano, G. N. (2004).

  Reproduction of the temperate octocoral *Tripalea clavaria*

- (Octocorallia, Anthothelidae) from sublittoral outcrops off Mar del Plata, Argentina. *Journal of the Marine Biological Association of the UK*, 84, 695–700. https://doi.org/10.1017/S0025315404009774h
- Excoffon, A. C., Belem, M. J. C., Zamponi, M. O., & Schlenz, E. (1997). The validity of Anthothoe chilensis (Actiniaria, Sagartiidae) and its distribution in southern hemisphere. *Iheringia Série Zoologia*, 82, 107-118.
- Falcâo, M., Santos, M. N., Drago, T., Serpa, D., & Monteiro, C. (2009). Effect of artificial reefs (southern Portugal) on sediment-water transport of nutrients: Importance of the hydrodynamic regime. Estuarine Coastal and Shelf Science, 83, 451–459. https://doi. org/10.1016/j.ecss.2009.04.028
- Farias, N. E., Obenat, S., & Goya, A. B. (2015). Outbreak of a neurotoxic side-gilled sea slug (*Pleurobranchaea* sp.) in Argentinian coasts. *New Zealand Journal of Zoology*, 42, 51–56.
- Farias, N. E., Wood, A. S., Obenat, S., & Schwindt, E. (2016). Genetic barcoding confirms the presence of the neurotoxic sea slug *Pleurobranchaea maculata* in southwestern Atlantic coast. *New Zealand Journal of Zoology*, 43, 292–298.
- Fox, J., & Weisberg, S. (2011). An R companion to applied regression, 2nd ed. Thousand Oaks CA: Sage. Retrieved from https://socserv.socsci.mcmaster.ca/jfox/Books/Companion.
- Fraschetti, S., Giangrande, A., Terlizzi, A., & Boero, F. (2003). Preand postsettlement events in benthic community dynamics. *Oceanologica Acta*, 25, 285–296. https://doi.org/10.1016/S0399-1784(02)01194-5
- Genzano, G. N. (2001). Associated fauna and sediment trapped by colonies of *Tubularia crocea* (Cnidaria, Hydrozoa) from the rocky intertidal of Mar del Plata, Argentina. *Biociencias*, *9*, 105–119.
- Genzano, G. N. (2002). Associations between pycnogonids and hydroids from the Buenos Aires littoral zone, with observations on the semi-parasitic life cycle of *Tanystylum orbiculare* (Ammotheiidae). *Scientia Marina*. 66, 83–92
- Genzano, G. N. (2005). Trophic ecology of a benthic intertidal hydroid, Tubularia crocea, at Mar del Plata, Argentina. Journal of the Marine Biological Association of the United Kingdom, 85, 307–312. https://doi.org/10.1017/S0025315405011197h
- Genzano, G. N. (2017) La vida en el mar. Buceando en la costa de Mar del Plata (2nd ed). Mar del Plata, Argentina (pp. 50): Instituto Nacional de Investigación y Desarrollo Pesquero, INIDEP.
- Genzano, G. N., Giberto, D., & Bremec, C. (2011). Benthic survey of natural and artificial reefs off Mar del Plata, Argentina, South-Western Atlantic. *Latin American Journal of Aquatic Research*, *39*, 553–566. https://doi.org/10.3856/vol39-issue3-fulltext-15
- Genzano, G. N., & San Martín, G. (2002). Association between the polychaete Procerastea halleziana (Polychaeta, Syllidae, Autolytinae) and the hydroid Tubularia crocea (Cnidaria, Hydrozoa) from the Mar del Plata intertidal zone, Argentina. Cahiers de Biologie Marine, 43, 165–170.
- Genzano, G. N., Zamponi, M. O., Excoffon, A. C., & Acuña, F. H. (2002). Hydroid populations from sublittoral outcrops off Mar del Plata, Argentina: Abundance, seasonality and reproductive periodicity. Ophelia, 56, 61-70. https://doi.org/10.1080/00785236.2002.104 09496
- Heery, E. C., Bishop, M. J., Critchley, L. P., Bugnot, A. B., Airoldi, L., Mayer-Pinto, M., ... Dafforn, K. A. (2017). Identifying the consequences of ocean sprawl for sedimentary habitats. *Journal of Experimental Marine Biology and Ecology*, 492, 31–48. https://doi.org/10.1016/j.jembe.2017.01.020
- Jimenez, C., Hadjioannou, L., Petrou, A., Andreou, V., & Georgiou, A. (2017). Fouling communities of two accidental artificial reefs (modern shipwrecks) in Cyprus (Levantine Sea). Water, 9, 11. https://doi.org/10.3390/w9010011
- Kühnemann, O. (1972). Bosquejo fitogeográfico de la vegetación marina del Litoral argentino. *Physis*, 31, 117-142.

- Lanfredi, N. W., Pousa, C. A., & Dragani, W. C. (1992). Wave-power potential along the coast of the province of Buenos Aires. *Energy*, 17, 997–1006.
- Lenth, R. V. (2016). Least-Squares Means: The R Package Ismeans. *Journal of Statistical Software*, 69(1), 1–33. https://doi.org/10.18637/jss. v069.i01
- Lessios, H. A. (1996) Methods for quantifying abundance of marine organisms. In: M. A. Lang, & C. C. Baldwin (Eds.), *Methods and techniques of underwater research* (pp. 149–157). Washington D.C.: Proceedings of the American Academy of Underwater Sciences Scientific Diving Symposium.
- Lozano-Cortés, D., & Zapata, F. (2014). Invertebrate colonization on artificial substrates in a coral reef at Gorgona Island, Colombian Pacific Ocean. *International Journal of Tropical Biology and Conservation*, 62, 161–168. https://doi.org/10.15517/rbt.v62i0.16273
- Meretta, P. E., & Genzano, G. (2015). Epibiont community variation on two morphologically different hydroid colonies: Amphisbetia operculata and Plumularia setacea (Cnidaria, Hydrozoa). Marine Biology Research, 11, 294–303.
- Meretta, P. E., Matula, C. V., & Casas, G. (2012). Occurrence of the alien kelp *Undaria pinnatifida* (Laminariales, Phaeophyceae) in Mar del Plata, Argentina. *Bioinvasions Records*, 1, 59–63. https://doi. org/10.3391/bir.2012.1.1.13
- Negri, R., Benavides, H. R., & Akselman, R. (2004). Algas del litoral marplatense. In E. E. Boschi, & M. B. Cousseau (Eds.), *La vida Entre Mareas: Vegetales y Animales de las costas de Mar del Plata* (pp. 73–86). Argentina: Publicaciones Especiales INIDEP.
- Nenadic, O., & Greenacre, M. (2007). Correspondence Analysis in R, with two- and three-dimensional graphics: The *ca* package. *Journal of Statistical Software*, 20, 1–13.
- Ninio, R., Delean, S., Osborne, K., & Sweatman, H. (2003). Estimating cover of benthic organisms from underwater video images: Variability associated with multiple observers. *Marine Ecology Progress Series*, 265, 107–116. https://doi.org/10.3354/meps265107
- Osborne, K., & Oxley, W. G. (1997) Sampling benthic communities using video transects. In: S. English, C. Wilkinson, & V. Baker (Eds.), Survey manual for tropical marine resources (pp. 363–376). Townsvile: Australian Institute of Marine Science.
- Ottaway, J. (1977). Pleurobranchaea novaezelandiae preying on Actinia tenebrosa. New Zealand Journal of Marine and Freshwater Research, 11, 125–130.
- Parma, A., Pascual, M., & Sar, E. (1987). Clave para el reconocimiento de los géneros de algas macrófitas del intermareal rocoso bonaerense. Serie técnica y didáctica (pp. 29). Argentina: Universidad Nacional de La Plata.
- Perkol-Finkel, S., Miloh, T., Zilman, G., Benayahu, S. I., & Benayahu, Y. (2006). Floating and fixed artificial reefs: The effect of substratum motion on benthic communities. *Marine Ecology Progress Series*, 317, 9–20.
- Perkol-Finkel, S., Shashar, N., Barneah, O., Ben-David-Zaslow, R., Oren, U., Reichart, T., ... Benayahu, Y. (2005). Fouling reefal communities on artificial reefs: Does age matter? *Biofouling*, 21(2), 127–140. https://doi.org/10.1080/08927010500133451
- R Development Core Team (2016). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/ [accessed on 11 April 2017].
- Rouse, G., & Pleijel, F. (2001). *Polychaetes* (pp. 354). Oxford: Oxford University Press.
- Seaman, W. J. E. (2000). Artificial reef evaluation: With application to natural marine habitats. CRC Marine Science Series (pp. 246). Boca Raton, FL: CRC Press.
- Silva, P. C., Woodfield, R. A., Cohen, A. N., Harris, L. H., & Goddard, J. H. R. (2002). First report of the Asian kelp *Undaria pinnatifida* in the Northeastern Pacific Ocean. *Biological Invasions*, 4, 333–338.

- Ulrich, I., Anger, K., & Schöttler, U. (1995). Tube-building in two epifaunal amphipod species, *Corophium insidiosum* and *Jassa falcata*. *Helgolander Wissenschaftliche Meersuntersuchungen*, 49, 393–398. https://doi.org/10.1007/BF02368364
- Willan, R. C. (1984). A review of diets in the Notaspidea (Mollusca: Opisthobranchia). *Journal of the Malacological Society of Australia*, 6, 125–142
- Zintzen, V., Massin, C., Norro, A., & Mallefet, J. (2006). Epifaunal inventory of two shipwrecks from the Belgian Continental Shelf. *Hydrobiologia*, 555, 207–219. https://doi.org/10.1007/s10750-005-1117-1
- Zintzen, V., Norro, A., Massin, C., & Mallefet, J. (2008). Spatial variability of epifaunal communities from artificial habitat: Shipwrecks in the Southern Bight of the North Sea. *Estuarine*

- Coastal and Shelf Science, 76, 327–344. https://doi.org/10.1016/j.ecss.2007.07.012
- Zuur, A. F., Ieno, E. N., Walter, N. J., Saveliev, A. A., & Smith, G. M. (2009). Mixed effects models and extensions in ecology with R (pp. 574). New York, NY: Springer.

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