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A preliminary assessment of larval fish assemblages on artificial reefs in the nearshore Southern Brazil

Cathrine Boerseth Alegretti[1], Henrique Grande[1,*], Cláudia Akemi Pereira Namiki[1], Robin Hilbert Loose[2], Frederico Pereira Brandini[1]

[1] Instituto Oceanográfico, Universidade de São Paulo (Praça do Oceanográfico, 191, 05508-120, São Paulo-SP, Brazil)
[2] Associação MarBrasil (Rua Ingá, 300, 83255-000, Pontal do Paraná-PR, Brazil)

* Corresponding author: henriquegrande@ymail.com

ABSTRACT

Artificial reefs (ARs) have been deployed on the inner shelf of Paraná, Southeast Brazil, as protection against destructive trawling activities, and to offer habitat for local fauna for recovery of biodiversity. The purpose of this study was to develop a preliminary characterization of the ichthyoplankton community associated with both artificial and natural reefs in the Currais Archipelago, and perform a comparison of fish larvae composition between ARs and nearby unconsolidated substrate. Two sampling methods were used, light traps and a plankton net. A total of 12 families and 14 species were identified, expanding the total species list in the area by eight species and three families. Differences among assemblages on ARs and unconsolidated substrate indicate that ARs seem to be effective attractors to fish larvae. Particular attention is drawn in regard to the "attractor effect" for exotic species that do not naturally reside in the area, such as *Omobranchus punctatus*. The large abundance of fish eggs on ARs suggests that these artificial structures can improve local production. These results are the first step needed to better define guidelines for sustainable use and management of ARs and Currais Archipelago, a Marine National Park.

Descriptors: Fish egg, Fish larvae, Light trap, Exotic species, Ecological succession.

INTRODUCTION

The bottom geomorphology of the Paraná state in Southern Brazil is dominated by mud and sand (Brandini and Silva, 2011) and since the late 1970s, its inner shelf ecosystems have suffered degradation, mainly related to commercial shrimp fisheries, including their associated bycatch and use of destructive fishing methods such as bottom trawling (Andriguetto et al., 2012). Artificial reef (AR) modules were first implanted in the area to act as anti-trawling units in 1997, and later, further units were installed between 2011 and 2012 in a line parallel to the coast (Brandini, 2014).

These ARs were also intended to offer habitat for local fauna, allowing the recovery of the biodiversity associated with natural reef habitats on the Paraná coast (Brandini and Silva, 2011; Brandini, 2014). In general, consolidated substrate is uncommon in the area, but provides rare rocky reef habitats, such as the ones found in the Currais Archipelago (a no-take Marine National Park established in 2013) and in Itacolomis Island. These environments thus enable high biomass and species diversity, with both

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commercial and ecological importance (Hackradt and Félix-Hackradt, 2009; Daros et al., 2012).

Most studies with ARs emphasize the community structure (Santos et al., 2010) and their great potential for aggregating, attracting and increasing fish biomass (Fujita et al., 1996; Osenberg et al., 2002; Cresson et al., 2014; Brochier et al., 2015). Like natural reef habitats, ARs are colonized by meroplanktonic larvae from the already existing larval pool in the water column; with time, colonizing organisms set the foundation for the development of a new biological community similar to that of natural rocky habitats nearby (Brandini and Silva, 2011; Cresson et al., 2014). In addition, ARs may allow marine species to spread over long distances through a series of dispersion events or short-distance colonization, acting as stepping stones or even corridors that facilitate the dispersion of both indigenous and non-indigenous species (McNeill et al., 2010; Airoldi et al., 2015), especially those associated with hard substrate habitats (Vaselli et al., 2008).

Aspects of early life stages are determinant in the dynamics and ecology of reef fishes (Beckerman et al., 2002), because many of the processes, such as transport, nutrition, growth and mortality (Houde, 2008), exert significant influence on population dynamics (Hellriegel, 2000; De Roos et al., 2003) and spatial distribution (Caley et al., 1996). However, previous studies of the ichthyofaunal assemblages associated with the Currais Archipelago, and ARs off the Paraná coast in general have focused on adult ichthyofauna (Hackradt and Félix-Hackradt, 2009; Daros et al., 2012), with early life stages of AR fishes still largely unknown.

Although the Paraná coast has been spatially restricted by protected areas and fisheries regulations, the absence of management plans (Faraco et al., 2016, de Oliveira Leis et al., 2019), presence of harbor activities (Katsumiti et al., 2009) and continued pressure on fisheries resources (de Oliveira Leis et al., 2019), have left the conservation status of marine ecosystems in the region under great concern. In this context, the present study aims to characterize and perform a preliminary analysis of the larval fish assemblages on ARs and natural reefs in the Currais Archipelago. Additionally, we compare the larval fish composition on ARs with that of nearby unconsolidated substrate as a first step to understand the development of fish assemblages, which is essential to support future coastal monitoring and conservation programs in the region

METHODS

STUDY AREA

The study area is located on the inner shelf of Paraná, Southeast Brazil (Figure 1), close to the Southern limit of the Brazilian Province, a zoogeographical province for western Atlantic shore fishes that stretches from the Amazon Delta down to Santa Catarina (Floeter and Gasparini, 2000).

The Paraná inner shelf is heavily influenced by sediments of terrestrial origin. The silt and clay percentage between the 5 and 15 m isobaths ranges from 10% up to 40% due to large quantities of fine particles in the continental runoff from the large estuarine bays of Paranaguá and Guaratuba (Brandini, 1990; Castro et al., 2006; Brandini and Silva, 2011;

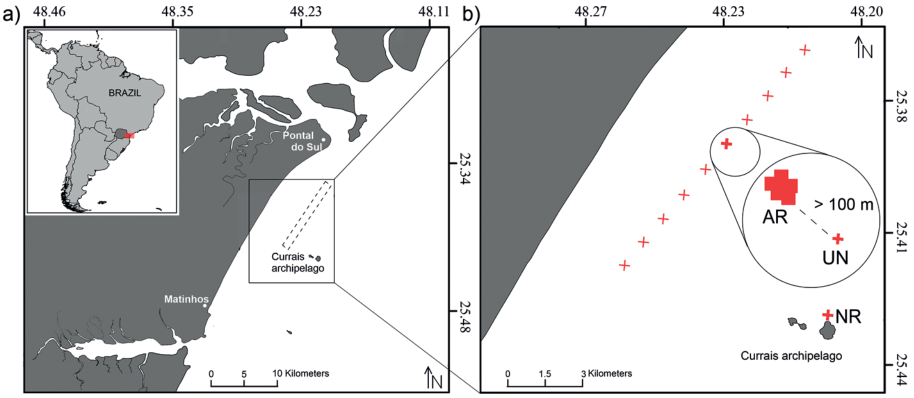

Figure 1. a) Study area off the northern coast of Paraná, Southern Brazil, b) location of Currais Archipelago, unconsolidated substrate and artificial reefs deployed by the REBIMAR project within the dotted rectangle.

Brandini, 2014). During winter, the region also receives terrigenous sediments from the La Plata river and Lagoa dos Patos estuary (Brandini, 1990). Bottom geomorphology is characterized by a lack of hard substrate. The few natural rocky habitats in the area are limited to bottom reefs in the Currais Archipelago (25°44′098″S; 48°21′752″W), Itacolomis Island (25°50′471″S; 48°24′638″W) and Figueira Island (25°21′384″S; 48°02′103″W). The Ilhas dos Currais Marine National Park was established in 2013 due to its economic and ecological importance, being a major commercial port and having an extensive system of estuaries with mangroves, covering an area of 1,359.7 hectares (Figure 1).

The hydrological structure of the inner shelf of Parana coast is basically dominated by winds (Noernberg et al., 2014). However, the influence of water masses is also a major oceanographic process in the dynamics of coastal and oceanic regions in south-southeast Brazil. Coastal Water (CW), with lower salinity (<34) and characterized by seasonal and geographical physical-chemical fluctuations (Castro et al., 2006), tends to predominate on the inner shelf (Nogueira and Brandini 2018). The outer shelf and offshore present a mixture of waters: Tropical Water (TW) and CW forming the upper layer and South Atlantic Central Water (SACW) at the bottom (Castro & Miranda, 1998; Nogueira and Brandini, 2018).

Between 2011 and 2012, 10 sets of concrete blocks were deployed in a line parallel to the coast, at the 12 m isobath within the framework of the Marine Biodiversity Recovery Program (REBIMAR in the Brazilian acronym) (Figure 1). Each set was composed of 300 to 400 grouped concrete blocks forming a vertical relief of approximately 2 m. The concrete blocks (composed of cement, natural sand, gravel, crushed stone and superplasticizer) have a four leaf clover-shaped hollow for increased surface area, an uneven surface to facilitate fixation of epilithic organisms and are enriched with silica to recreate the pH value of natural rocky reefs (Figure 2).

Sampling

Three sampling areas were selected to characterize the larval fish assemblage: I) the 5th AR located in the middle of the REBIMAR line (25°40′30″S, 48°23′47″W), at depths ranging from 10 to 12 m; II) an area of unconsolidated substrate (UN) comprising a flat sandy-mud bottom located at a distance greater than 100 m from the ARs (25°40′33″S, 48°24′07″W)

Figure 2. Concrete blocks used as ARs in the REBIMAR project.

with depths approximately the same as the AR; and III) the nearest natural rocky reef (NR) located approximately 5,000 m from the ARs, on the northern portion of the Currais Archipelago (25°44′01″S, 48°21′55″W) and at depths up to approximately 8 m (Figure 1).

Two methods were used to sample fish eggs and larvae and obtain a more complete view of the ichthyoplankton assemblage: 1) light traps and 2) plankton tows using a net attached to an underwater scooter adapted from Beldade et al. (2006) and Borges et al. (2007).

Light traps used in this study were made from repurposed polyethylene terephthalate (PET) containers (20 L), with a 5 W 10 mm LED light system (n = 8) and a 6 V battery within a waterproof cylinder in the center. Eight openings were cut symmetrically around the PET container and corresponding LED lights were positioned within the waterproof cylinder so that each light pointed directly to an opening in the trap. The LED lights were controlled with a magnetic reed switch. Light traps were held in suspension in the water column between a bottom anchor and a mid-depth buoy; a separate cable connected the anchor to a surface buoy. The mid-depth buoy provided enough buoyancy for the light traps to remain in a vertical position (Figure 3). Light traps were deployed in the afternoon and recovered as early as possible the following day. One light trap was placed semi-simultaneously above the AR, UN and NR in each sampling excursion.

Tow sampling was performed with a plankton net attached to an underwater scooter (Seadoo GTS 30M) and manually controlled by a scuba diver at a speed of approximately 1.5 knots. The plankton net had a mesh size of 350 µm, 0.30 m mouth diameter and a diameter/length ratio of 1:3. A flowmeter (LUNUS 2030R) attached to the mouth of the net was used to obtain the volume of filtered water. Each tow extended from the surface to close to the reefs or unconsolidated substrate, where was it maintained at a distance of approximately 1 m from the substrate for approximately 5 minutes, after which the diver would ascend to the surface. This method was chosen for its maneuverability and capability for collection in proximity to bottom and complex structures such as rocky reefs or AR modules (Beldade et al., 2006; Borges et al., 2007). One tow was performed in proximity to the AR, UN and NR during each sampling excursion.

In total, 11 sampling excursions were conducted from July 2014 to April 2016, over the course of which 15 individual samples were collected with a light trap

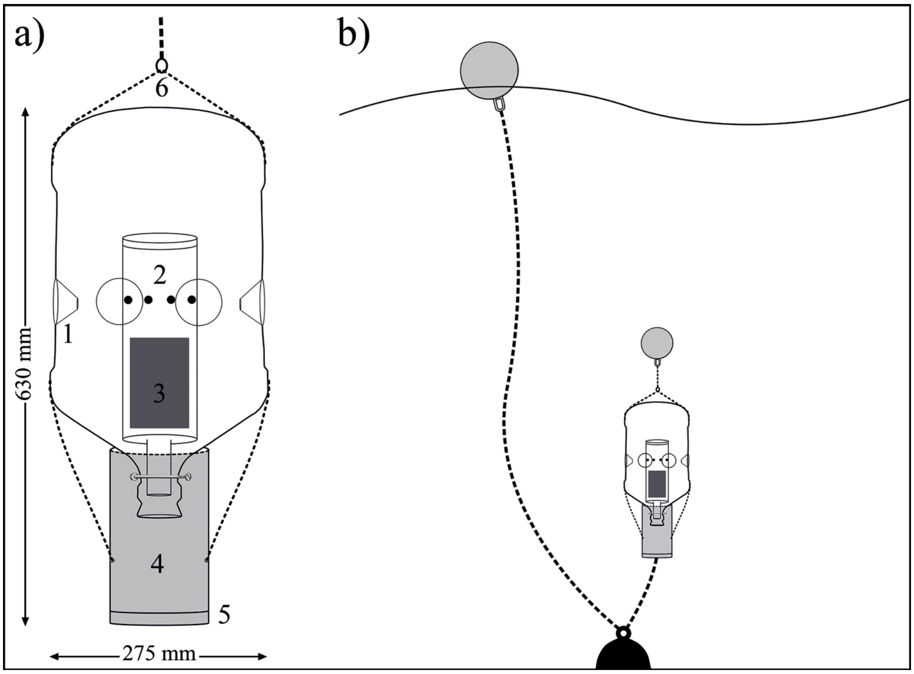

Figure 3. a) Light trap made from repurposed PET bottle: 1. entrance; 2. LED lighting system; 3. battery within waterproof container; 4. Container; 5. 350 µm mesh; 6. cable. b) Visual representation of light trap suspended in the water column between bottom anchor and mid-depth buoy.

(AR and UN = 6, NR = 3) and 22 samples with the plankton net (AR and UN = 9, NR = 4). For operational purposes we used the austral seasons as: summer (January to March), fall (April to June), winter (July to September) and spring (October to December). Sampling was originally intended to be conducted monthly, but access to the locations was limited by weather conditions and mechanical failures that precluded boat field-work. This resulted in a low sample number (especially in the Currais Archipelago – 12 Km from the coast) in periods with high wind or rough seas.

LABORATORY PROCEDURE

Samples were preserved in buffered 4% formalin. In the laboratory, fish eggs and larvae were separated and counted under a stereomicroscope. Larval fishes were transferred to 70% alcohol. Meristic (e.g. number of rays and spines in the fins) and morphometric characteristics (e.g. body shape, relative position of the fin) were used to identify individuals to the lowest possible taxonomic level according to Matsuura (1977), Whitehead et al. (1988), Leis and Carson-Ewart (2000) and Richards (2006). Developmental stage of the fish larvae was classified using the flexion of the tip of the notochord, into preflexion, flexion or postflexion stage (Kendall et al., 1984).

CHARACTERIZATION OF FISH LARVAE

Identified taxa were also classified within the following categories: Rocky Reef (RR), taxa that are found on and are characteristic of reefs (i.e. the consensus list *sensu* Bellwood 1998, plus available published manuscripts on reef species in this region); Sandy Bottom (SB), taxa living closely associated with soft bottom substrate; and Pelagic (PLG), taxa occurring in the water column. Classification was done according to the preferred habitat of adult fishes following published research (Richards, 2006; Leis and Carson-Ewart, 2000; Bellwood and Wainwright, 2002; Froese and Pauly, 2016).

Fish species composition at the sampling sites (AR, UN and NR) was compared to juveniles and adult fishes recorded in the area (Pinheiro, 2005; Hackradt and Félix-Hackradt, 2009; Hackradt et al., 2011; Daros et al., 2012; Santos, 2014; Daros et al., 2018) (Table1), to obtain a more precise list of species occurring in the region.

DATA ANALYSES

Before the statistical analyses were carried out, samples from the Currais Archipelago (NR) and spring, autumn, and winter data were excluded from the analyses due to inadequate level of information (low sample number). Owing to multi-year summer sampling (2014 to 2016), an analysis of similarity (ANOSIM) (Clark and Warwick, 2001) was carried out to test differences in the larval fish assemblage between years. Because all year terms were non-significant ($R = 0.019$, $P > 0.05$), we pooled data from all sampling periods for further analysis.

A permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was next performed to examine differences between AR and UN samples. The Jaccard similarity index was used for larval fish assemblage (presence-absence transformed data) and Euclidean distance for larval richness, total abundance of larvae, total abundance of eggs and abundance of larval stage ($\log(x + 1)$ transformed data). The statistical analysis for total abundance of larvae was performed separately for each sampling method due to the absence of comparable metric units (light trap - individuals/sampling and plankton net – individuals m^{-3}). The test was done using 9999 permutations under the reduced model. The PERMANOVA pairwise tests were performed to examine differences between sampling sites.

To summarize multivariate patterns in the larval fish assemblage between AR and UN, a canonical analysis of principal coordinates (CAP) was performed (Anderson and Willis, 2003). A pairwise comparison of similarity percentages (SIMPER) was used to indicate which larval fish species contributed the most to assemblage differences between these sites. A 1000-interations SIMPER, identified the percentage contribution of each taxon to the dissimilarity between AR and UN (Clarke, 1993). All multivariate analyses were performed using PRIMER v6 with the PERMANOVA + software package.

RESULTS

TAXONOMIC COMPOSITION

A total of 277 fish larvae, encompassing 12 families, 14 genera and 12 species were collected (Table 1). Approximately 8.7% of larvae were only identified

Table 1. Families and species on natural and artificial reef habitats off Paraná, Brazil. Families are listed in phylogenetic order according to Nelson et al. (2016). Genera and species are alphabetically ordered. Source references are as follows: Pinheiros (2005), Hackradt and Félix-Hackradt (2009), Hackradt et al. (2011), Daros et al. (2012), Santos (2014) and Daros et al. (2018). Labels: RAM = Artificial reef deployed in 1997; REBIMAR = Artificial reef deployed between 2011 and 2012; Cur = Currais Archipelago; Ita = Itacolomis Island; AR = Artificial reef (REBIMAR); UN = Unconsolidated substrate; NR = Natural rocky reef (Currais Archipelago); PH = preferential habitat: RR = rocky reef; SB = sandy bottom; SB/RR = sandy bottom / rocky reef; PLG = pelagic; NA = not available.

Family/Species	**Adult fish**			**Larval fish**			**PH**
	RAM	REBIMAR	Cur/Ita	(AR)	(UN)	(NR)	
Myliobatidae							
Aetobatus narinari (Euphrasen, 1790)			X				PLG
Muraenidae							
Gymnothorax funebris (Ranzini, 1840)			X				RR
Gymnothorax moringa(Cuvier, 1829)	X		X				RR
Gymnothorax vicinus (Castelnau, 1855)	X		X				RR
Ophichthidae							
Myrichthys breviceps (Richardson, 1848)		X	X				SB/RR
Myrichthys ocellatus (Lesueur, 1825)			X				SB/RR
Engraulidae							
Engraulis anchoita (Hubbs & Marini, 1935)				X	X	X	PLG
Engraulidae sp.				X	X		PLG
Clupeidae							
Harengula clupeola (Cuvier, 1829)	X		X	X	X	X	PLG
Sardinella brasiliensis (Steindachner, 1879)	X		X			X	PLG
Synodontidae							
Synodus foetens (Linnaeus, 1766)			X			X	SB/RR
Synodus synodus (Linnaeus, 1758)			X				SB/RR
Ogcocephalidae							
Ogcocephalus vespertilio (*Linnaeus*, 1758)			X				RR
Hemiramphidae							
Hemiramphus brasiliensis (Linnaeus, 1758)	X		X				PLG
Hyporhamphus unifasciatus (Ranzani, 1842)	X		X				PLG
Holocentridae							
Holocentrus adscensionis (Osbeck, 1795)	X		X				RR
Synghnathidae							
Halicampus crinitus (Jenys, 1842)			X				RR
Hippocampus reidi (Ginsburg, 1933)	X						RR
Micrognathus crinitus (Jenys, 1842)			X				RR
Pseudophallus mindii (Meek & Hildebrand, 1923)			X				SB/RR
Fistulariidae							
Fistularia tabacaria (Linnaeus, 1758)	X		X				RR
Dactylopteridae							
Dactylopterus volitans (Linnaeus, 1758)			X				SB
Scorpaenidae							
Scorpaena brasiliensis (Cuvier, 1829)			X				RR

Continued Table 1.

Scorpaena plumieri (Bloch, 1793)	X		X					RR
Triglidae								
Prionotus punctatus (Bloch, 1792)	X							SB
Centropomidae								
Centropomus undecimalis (Bloch, 1792)	X	X						SB
Serranidae								
Diplectrum radiale (Quoy and Gaimand, 1824)	X	X	X					SB
Serranus atrobranchus (Cuvier, 1829)	X							SB
Serranus flaviventris (Cuvier, 1829)	X		X					RR
Rypticus randalli (Courtenay, 1967)		X						SB/RR
Rypticus saponaceus (Bloch & Schneider, 1801)			X					
Epinephelidae								
Epinephelus adscensionis (Osbeck, 1765)			X					RR
Epinephelus itajara (Lichtenstein, 1822)	X	X	X					RR
Epinephelus marginatus (Lowe, 1834)		X						RR
Epinephelus morio (Valenciennes, 1828)			X					SB/RR
Hyporthodus niveatus (Valenciennes, 1828)	X		X					SB/RR
Mycteroperca acurtirostris (Valenciennes, 1828)	X	X	X					RR
Mycteroperca bonaci (Poey, 1860)			X					RR
Mycteroperca interstitialis (Poey, 1860)		X	X					RR
Mycteroperca marginata (Lowe, 1834)	X		X					RR
Mycteroperca microleps (Goode & Bean, 1880)	X							SB/RR
Mycteroperca venenosa (Linnaeus, 1758)			X					RR
Priacanthidae								
Priacanthus arenatus Cuvier, 1829		X	X					RR
Pomatomidae								
Pomatomus saltatrix (Linnaeus, 1766)	X							PLG
Echeneidae								
Echeneis naucrates (Linnaeus, 1758)	X							PLG
Rachycentron canadum								
Rachycentron canadum (Linnaeus, 1766)	X							PLG
Carangidae								
Carangoides crysos (Mitchill, 1815)	X	X	X					PLG
Caranx hippos (Linnaeus, 1766)		X						PLG
Caranx latus (Agassiz, 1831)	X		X					PLG
Carangoides ruber (Bloch, 1793)					X			PLG
Chloroscombrus chrysurus (Linnaeus, 1766)	X	X	X		X	X	X	SB/RR
Oligoplites saliens (Bloch, 1793)	X		X					PLG
Pseudocaranx dentex (Bloch & Schemeider, 1801)	X		X					RR
Seriola rivoliana (Linnaeus, 1758)	X							PLG

Continued Table 1.

Selene setapinnis (Mitchill, 1815)			X					PLG
Selene volmer (Linnaeus, 1758)			X					PLG
Trachinotus carolinus (Linnaeus, 1766)		X						PLG
Trachinotus falcatus (Linnaeus, 1758)			X					PLG
Lutjanidae								
Lutjanus analis (Cuvier, 1828)	X	X	X					SB/RR
Lutjanus jocu (Block & Schneider, 1801)			X					SB/RR
Lutjanus synagris (Linnaeus, 1758)	X							SB
Ocyurus chrysurus (Bloch, 1791)			X					RR
Rhomboplites aurorubens (Cuvier, 1828)	X							RR
Gerreidae								
Diapterus rhombeus (Cuvier, 1829)	X	X					X	SB
Eucinostomus argenteus (Baird & Girard, 1855)	X							SB
Haemulidae								
Anisotremus surinamensis (Bloch, 1791)	X	X	X					RR
Anisotremus virginicus (Bloch, 1791)	X		X					RR
Haemulon aurolineatum (Cuvier, 1830)	X	X	X				X	SB/RR
Haemulon steindachneri (Jordan & Guilbert, 1882)	X		X					SB/RR
Orthopristis ruber (Cuvier, 1830)	X	X	X					SB/RR
Conodon ruber (Cuvier, 1830)		X						
Sparidae								
Archosargus probatocephalus (Walbaum, 1792)	X	X	X					RR
Archosargus rhomboidalis (Linnaeus, 1758)		X						RR
Calamus Penna (Valenciennes, 1830)			X					SB/RR
Calamus pennatula (Guichenot, 1868)	X							SB
Diplodus argenteus (Valensiennes, 1830)	X	X	X					RR
Scianidae								
Bairdiella ronchus(Cuvier, 1830)							X	SB
Cynoscion jamaicensis (Vaillant & Bocourt, 1883)			X					SB
Odontoscion dentex (Cuvier, 1830)	X		X					SB/RR
Paraques acuminatus (Bloch & Schneider, 1801)	X		X					SB/RR
Scianidae sp.					X	X		NA
Mullidae								
Pseudupeneus maculatus (Bloch, 1793)	X		X					SB/RR
Pempheridae								
Pempheris schomburgkii (Müller & Troschel, 1848)			X					RR
Kyphosidae								
Kyphosus sp.			X					RR
Chaetodontidae								
Chaetodon striatus (Linnaeus, 1758)	X		X					RR

Continued Table 1.

Chaetodon striatus (Linnaeus, 1758)	X		X					RR
Pomacanthidae								
Holocanthus tricolor (Bloch, 1795)			X					RR
Pomachantus paru (Bloch, 1787)	X		X					RR
Pomacentridae								
Abudefdud saxatilis (Linnaeus, 1758)	X	X	X					RR
Chromis multilineata (Guichenot, 1853)			X					RR
Stegastes fuscus (Cuvier, 1830)			X					RR
Stegastes pictus (Castelnau, 1855)			X					RR
Stegastes variabilis (Castelnau, 1855)	X		X					RR
Labridae								
Bodianus pulchellus (Poey, 1860)			X					RR
Bodianus rufus (Linnaeus, 1758)			X					RR
Cryptotomus roseus (Cope, 1871)			X					
Halichoeres cyanocephalus (Bloch, 1791)			X					RR
Halichoeres poeyi (Stendachner, 1867)	X		X					SB/RR
Xyrichtys novacula (Linnaues, 1758)			X					RR
Labridae - Scarinae								
Nicholsina usta (Valenciennes, 1836)			X					RR
Sparisoma amplum (Ranzani, 1842)			X					RR
Sparisoma axillare (Steindachner, 1878)	X		X					RR
Sparisoma chrysopterum (Bloch & Schneider, 1801)			X					RR
Sparisoma frondosum (Agassiz, 1831)			X					RR
Sparisoma radians (Valenciennes, 1840)			X					RR
Sparisoma rubripinne (Valenciennes, 1840)			X					RR
Labrisomidae								
Labrisomus sp.							X	RR
Labrisomus nuchipinnis (Quoy & Gaimand, 1824)	X		X					RR
Malacoctenus delalandii (Valenciennes, 1836)	X	X	X					RR
Paraclinus spectator (Guimarães & Bacellar, 2002)			X					RR
Starksia brasiliensis (Gilbert, 1900)			X					RR
Chaenopsidae								
Emblemariops signifera (Ginsburg, 1942)	X		X					RR
Dactyloscopidae								
Dactyloscopidae sp.							X	SB
Blenniidae								
Hypsoblennius invemar (Smith-Vaniz & Acero, 1980)	X		X					RR
Parablennius marmoreus (Poeu, 1876)	X		X					RR
Parablennius pilicornis (Cuvier, 1829)	X		X					RR
Scartella cristata (Linnaeus, 1758)			X					RR

Continued Table 1.

Omobranchus punctatus (Valenciennes, 1836)					X		X	RR
Ophioblennius sp.			X					RR
Gobiidae								
Bathygobius soporator (Valenciennes, 1837)			X					RR
Coryphopterus glaucofraenum (Gill, 1863)	X		X				X	SB/RR
Ctenogobius boleosoma (Jordan & Gilbert, 1882)			X					SB/RR
Ephippidae								
Chaetodipterus faber (Broussonet, 1782)	X	X	X					PLG
Achanturidae								
Achanturus bahianus (Castelnau, 1855)			X					RR
Acanthurus chirurgus (Bloch, 1787)	X		X					RR
Acanthurus coeruleus Bloch & Schneider, 1801			X					RR
Sphyraenidae								
Sphyraena guachancho (Cuvier, 1829)	X							PLG
Sphyraena tome (Fowler, 1903)	X		X					PLG
Trichiuridae - Trichiurinae								
Trichiurus lepturus (Linnaeus, 1758)					X	X		PLG
Scombridae								
Scomberomorus brasiliensis (Collete, Russo & Zavala-Camin, 1978)	X		X					PLG
Scomberomorus cavalla (Cuvier, 1829)	X							PLG
Paralichthyidae								
Paralichthys sp.		X						SB
Bothidae								
Bothus ocellatus (Agassiz, 1831)			X					SB
Balistidae								
Balistes capriscus (Gmelin, 1789)	X	X						SB/RR
Monocanthidae								
Stephanolepis hispidus (Linnaeus, 1766)		X	X					SB/RR
Ostraciidae								
Acanthostracion quadricornis (Linnaeus, 1758)			X					SB/RR
Tetraodontidae								
Sphoeroides greeleyi (Gilbert, 1900)			X					RR
Sphoeroides spengleri (Bloch, 1785)			X					SB/RR
Sphoeroides testudineus (Linnaeus, 1758)			X					SB/RR
Diodontidae								
Chilomycterus spinosus (Linnaeus, 1758)			X					SB/RR

to the family level while 5.4% of larvae were unidentifiable because of significant structural damage or a lack of identifiable characteristics. Out of the 272 fish eggs collected, seven were elliptical and identified as belonging to the family Engraulidae.

Fish larvae of all developmental stages were found in all sampling sites. Although there are no significant differences in the abundance of larval stages between AR and UN (Table 2), the proportions of larvae at different development stages varied according to sampling method. In plankton net samples the predominant larval stages were preflexion (90.1%) (Figure 4a). In light trap samples, postflexion larvae accounted for 95% of the total, while only 3% were in the flexion stage (Figure 4b). Fish eggs were only present in plankton net samples.

The most abundant fish larvae families collected on the AR were Carangidae (69%) and Engraulidae (26%) (Figure 5a). At the species level, the most abundant were the bar jack *Caranx ruber* (Bloch, 1793) and

Table 2. PERMANOVA based on the Jaccard similarity index for larval fish assemblage (presence-absence transformed data) and Euclidean distance for larval fish richness, abundance (light trap and plankton net), total abundance of eggs and abundance of larval stages (preflexion, flexion, and postflexion).

Larval Fish Assemblage						
Source	*df*	SS	MS	*Pseud-F*	perms	*P (MC)*
Habitats	1	4140	4140	2.61	307	0.039
Residue	18	28464	1581			
Larval Fish Richness						
Source	*df*	SS	MS	*Pseud-F*	perms	*P (MC)*
Habitats	1	2.96	2.96	7.13	48	0.017
Residue	18	7.48	0.41			
Larval Fish Abundance (Light trap)						
Source	*df*	SS	MS	*Pseud-F*	perms	*P (MC)*
Habitats	1	12.92	12.92	1.42	11	0.063
Residue	6	54.52	9.08			
Larval Fish Abundance (Plankton net)						
Source	*df*	SS	MS	*Pseud-F*	perms	*P (MC)*
Habitats	1	3.32	3.32	4.81	9	0.057
Residue	10	6.91	0.69			
Total Abundance of Eggs						
Source	*df*	SS	MS	*Pseud-F*	perms	*P (MC)*
Habitats	1	7.68	7.86	6.72	748	0.013
Residue	18	21.05	1.17			
Abundance of preflexion larvae stage						
Source	*df*	SS	MS	*Pseud-F*	perms	*P (MC)*
Habitats	1	2.72	2.72	3.85	16	0.071
Residue	18	9.33	0.52			
Abundance of flexion larvae stage						
Source	*df*	SS	MS	*Pseud-F*	perms	*P (MC)*
Habitats	1	0.45	0.45	1.97	8	0.194
Residue	18	4.1	0.23			
Abundance of postflexion larvae stage						
Source	*df*	SS	MS	*Pseud-F*	perms	*P (MC)*
Habitats	1	3.96	3.96	0.91	12	0.366
Residue	18	77.19	4.31			

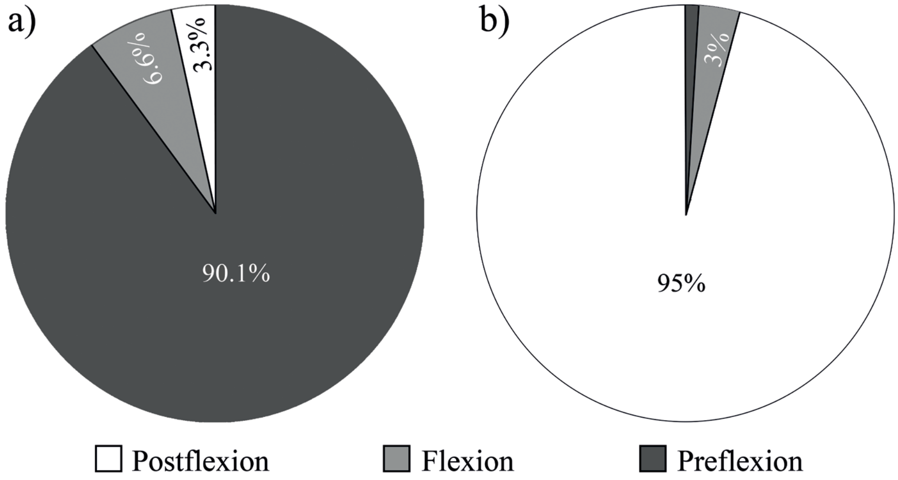

Figure 4. Proportions of larvae at different development stages in a) plankton net samples and b) light trap samples.

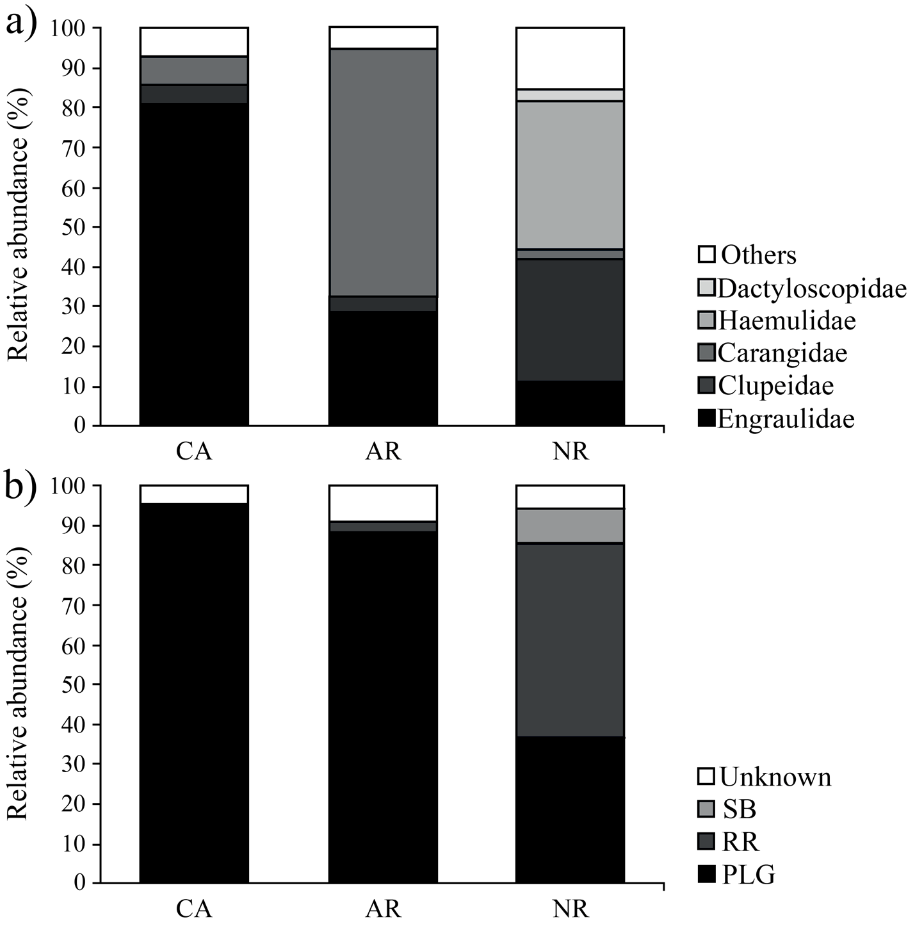

Figure 5. a) Relative abundance of dominant families collected; b) Proportions of preferred adult habitat among sampled larvae. Labels: RR = rocky reef; PLG = pelagic; SB = soft bottom; UN = unconsolidated substrate; AR = artificial reef; NR = natural rocky reef.

the Argentine anchovy *Engraulis anchoita* (Hubbs and Marini 1935). The most abundant families collected in the UN area were Engraulidae (80%) and Carangidae (8%). At the species level, *Engraulis anchoita* and the Atlantic bumper *Chloroscombrus chrysurus* (Linnaeus, 1766) were the most abundant species. Most larvae collected on the UN and AR were pelagic, representing 96% and 89% of individuals, respectively (Figure 5b).

In samples from the NR, the most abundant families were Haemulidae (35%), Clupeidae (32%) and Engraulidae (10%). At the species level, the most abundant were the tomtate grunt *Haemulon aurolineatum* (Cuvier 1830), scaled herring *Harengula clupeola* (Poey, 1865) and *Engraulis anchoita.* Most larvae collected on the NR were characteristic of rocky reefs (57%), followed by pelagic (46%) and soft bottom species (10%) (Figure 5b).

Eight species (*Bardiella ronchus*, *Carangoides ruber*, *Engraulis anchoita*, *Harengula clupeola*, *Omobranchus punctatus*, *Sardinella brasiliensis*, *Trichiurus lepturus* and Dactyloscopidae sp.) and three families (Dactyloscopidae, Engraulidae and Trichiuridae) collected in this work were not listed in previous studies of adult fishes from Currais Archipelago or on ARs in the region (Hackradt and Félix-Hackradt, 2009; Daros et al., 2012; Daros et al., 2018) including REBIMAR (Santos, 2014) (Table 1).

Two individuals of the invasive muzzled blenny *Omobranchus punctatus* (Valenciennes, 1836) in the postflexion stage were collected in two different light trap samples. One larva (18.76 mm standard length) was captured on the AR on February 2015 and one larva (9.81 mm standard length) was captured on the NR on March 2015.

COMPARISON BETWEEN ARTIFICIAL REEF AND UNCONSOLIDATED SUBSTRATE

The larval fish assemblage showed a significant difference between AR and UN samples (P = 0.039, PERMANOVA; Table 2). A CAP illustrates differences in larval fish assemblage between AR and UN samples (Figure 6). The success percentages in sample classification (70% samples correctly classified) and high canonical correlations (δ2 CAP1 = 0.39; δ2 CAP2 = 0.52) confirm high distinction in groups between the assemblages of fish larvae in artificial reefs from unconsolidated habitats. However, the CAP plot did not show distinct groups between the sampled methods (light trap and plankton net) (Figure 6).

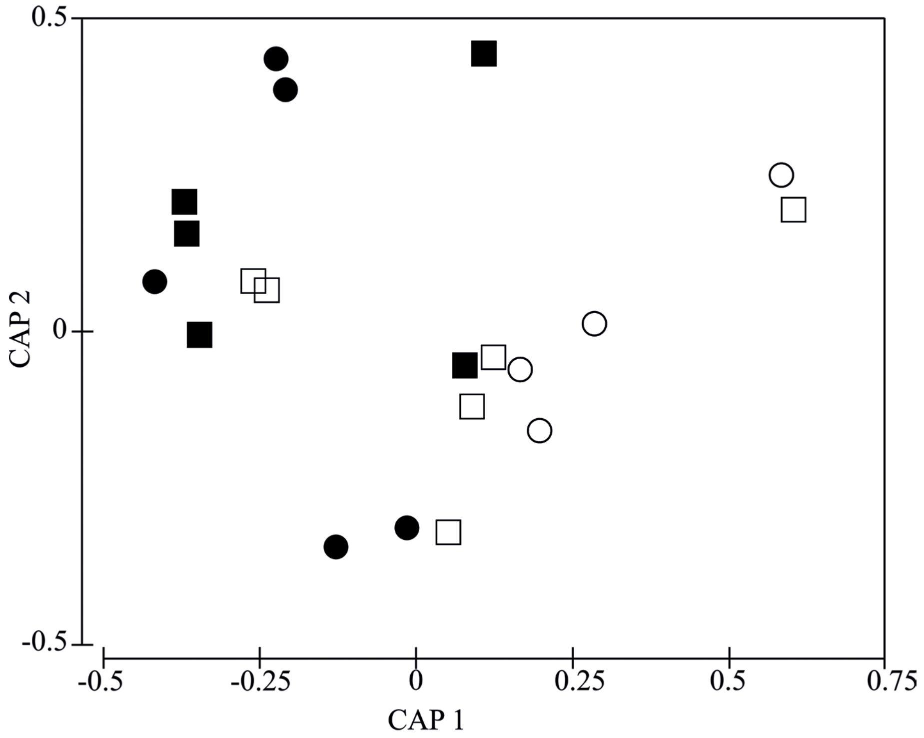

Figure 6. A CAP ordination plot showing the relationship of larval fish assemblages between artificial reef (black) and unconsolidated substrate (white) samples caught by light traps (circle) and plankton net (square).

Larval fish richness and abundance of eggs were significantly higher in AR samples compared to UN (Table 2; Figure 7a, b). In contrast, abundance of larvae (light trap and plankton net samples) showed trends toward a high number on the AR, although they were not statistically significant (Table 2; Figure 7c, d).

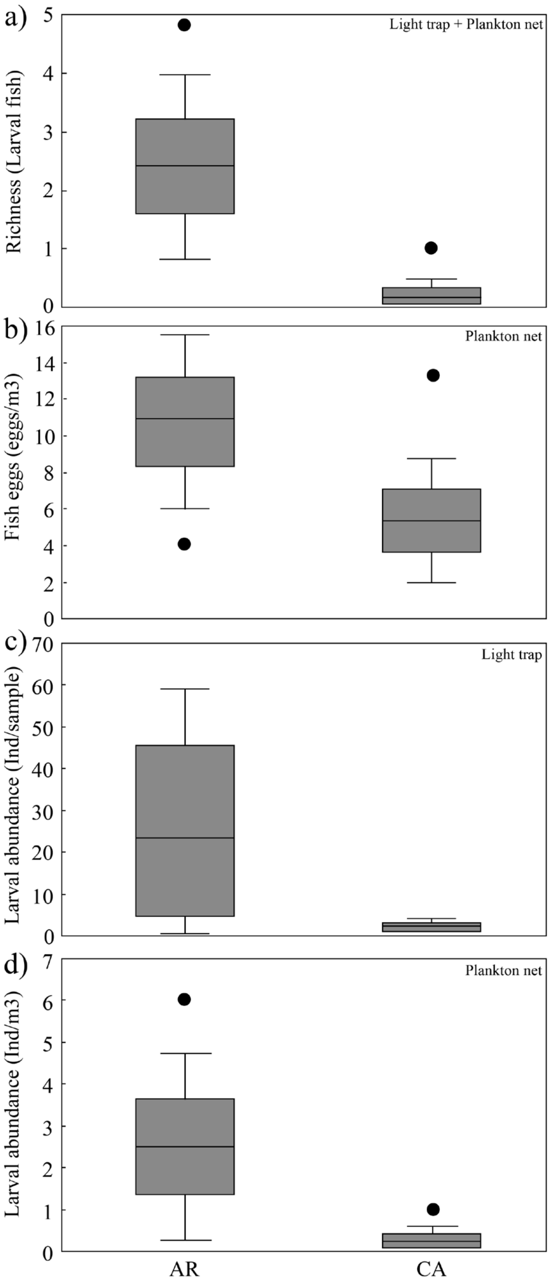

Figure 7. Box and whisker plots of a) larval fish richness, b) total abundance of fish larvae sampled by light traps, c) total abundance of fish larvae sampled by plankton net and d) total abundance of fish eggs. Middle lines represent mean values and boxes are mean ± standard error. Whiskers represent mean ± standard deviation. Dots show outlier variation. AR = artificial reef; UN = unconsolidated substrate.

SIMPER analysis revealed that average dissimilarity between AR and UN samples was 89.7% (Table 3). The rocky reef fish *Omobranchus punctatus*, pelagic/reef-associated *Caranx ruber* and *Chloroscombrus chrysurus*, pelagic *Engraulis anchoita* and Sciaenidae sp. were responsible for 86% of total dissimilarity between AR and UN (Table 3). Among these species, *C. ruber, O. punctatus*, and Sciaenidae sp. were found only in summer AR samples.

DISCUSSION

The ARs deployed on unconsolidated substrate off the coast of Paraná seem to be acting as attractors to fish larvae. In addition, a large abundance of fish eggs on the ARs compared to UN suggests that these ARs are evolving into a productive marine habitat (source area). It is important to note that the highlighted results refer exclusively to the summer period. Therefore, it should not be assumed that ichthyoplankton (richness and abundance) are temporally stable since several other studies have shown a clear temporal variability (Nonaka et al., 2000; Castro et al., 2005; Macedo-Soares et al., 2009).

High abundance and richness of fish larvae and presence of pelagic engraulids, carangids and clupeids on the ARs, are indicative of the attraction effect exerted by hard substrate in relation to the extensive flat unconsolidated mud and sand bottom substrate in the surrounding area. These groups of pelagic species are probably attracted by the larger structure and vertical relief ARs (Walsh, 1985), with reef modules offering valuable protection and feeding grounds for larvae during their most vulnerable early life-stages (Davis et al., 1982).

Greater abundance of eggs and yolk-sac larvae in AR samples compared to UN are important evidence that this new habitat has potential for increasing fish production in the study area. In this sense, the habitat created by artificial hard substrate may facilitate reproduction on or near ARs. Artificial reefs may play an ecologically important role in coastal systems, and in some cases can enhance local-regional eggs and larvae and biomass production (Campos and Gamboa, 1989; DeMartini et al., 1994; Stephens and Pondella, 2002; Andersson and Öhman, 2010).

Table 3. Results of SIMPER analysis comparing larval fish assemblages on the artificial and unconsolidated substrate. Species that contributed the most toward average dissimilarity between different sampling areas are listed. Labels: Cum. Contrib. - Cumulated Contribution.

Species	Dissimilarity (%)	Cum. Contrib. (%)
Comparison between AR and UN (average similarity: 10.3%; average dissimilarity: 89.7%)		
Caranx ruber	53.84	53.84
Chloroscombrus chrysurus	13.58	67.42
Omobranchus punctatus	8.03	75.45
Engraulidae sp.	6.02	81.47
Sciaenidae sp.	4.85	86.32

Despite the 'attraction versus production' debate (Bohnsack, 1989; Lindberg, 1997), production and attraction hypothesis are not necessarily mutually exclusive, but rather can be viewed as two extremes along a gradient (Svane and Petersen, 2001). In addition to fish aggregation, there was evidence of increase in biomass and ongoing recruitment through reproduction. However, because larvae produced on a reef are generally widely distributed through a dispersive pelagic phase, with few species showing larval retention, an analysis of larval recruitment from surrounding areas is necessary to prove increased production on ARs.

Fish colonization on marine artificial reefs can reach a stable assemblage structure over periods ranging from two months (Cummings, 1994; Golani and Diamant, 1999) to more than five years (Bohnsack et al., 1991). The presence of both larval and adult stages (Table 1) of reef-associated species and intermediate stages on ARs indicates that these may be in a transitional phase of an ecological succession process. Despite artificial reefs previously deployed off Paraná being susceptible to colonization (Hackradt et al., 2011), species composition may not resemble natural communities (Svane and Petersen, 2001). The main dissimilarity in larval fish assemblages between AR and UN, was due to the presence of some species at the interface of pelagic/reef environments, such as the carangids *Caranx ruber* and *Chloroscombrus chrysurus*. Carangids do not have a clear division between pelagic and demersal phases; although they do not settle in reef habitats (Catalán et al., 2014), they are common in clear island areas or adjacent to coral reefs off mainland coasts and considered both pelagic and reef-associated as adults (Cervigón et al., 1992). In addition, the exotic Indo-Pacific muzzled blenny *Omobranchus punctatus* (Costa et al., 2011; Contente et al., 2015), a species characteristic of rocky reefs, was recorded for the first time on an AR and in Paraná. *Omobranchus punctatus* is a coastal and brackish water species that lives in cryptic benthic habitats. The cryptic nature causes it to seek refuge in small crevices, such as ballast-intake holes in ship hulls (Gerhardinger, 2006).

The presence of the blenny *O. punctatus* indicates that ARs can also attract fish species that would not naturally reside in the area (Wilhelmsson et al., 2006), hence potentially acting as corridors or stepping stones, enhancing the spread of invasive species (Airoldi et al., 2015). An example is the lionfish from the Indo-Pacific region. Despite being strongly associated with natural rocky or coral reefs (Côté and Maljkovic, 2010), when invading areas with an unconsolidated substrate (e.g. estuaries), these fish are often found associated with artificial structures (Jud et al., 2011). However, evidence linking artificial reefs and the spread of alien species over a specific geographic region is lacking and requires further investigation.

Although the present work recorded a low representation of reef fish larvae on ARs, a previous environmental monitoring study of juveniles and adults reported the presence of typical species of reef fishes, such as Haemulidae (*Haemulon aurolineatum, Orthopristis ruber, Anisotremus surinamensis*), Epinephilidae (*Epinephelus itajara, Epinephelus marginatus, Mycteroperca acutirostris*), Pomacentridae (*Abudefduf saxatilis*) and Labrisomidae (*Malacoctenus delalandii*) (Santos, 2014) (Table 1). Obviously, this begs the question as to why reef fish larvae were absent or underestimated in the present study.

The low frequency and absence of some reef fish species on ARs may result from a range of ecological processes and life history strategies (Gaston, 1994; Martin et al., 2005). It is possible that species not observed on ARs, such as those hatching from demersal eggs, show considerable larval retention (Jones et al., 2005). For example, the pomacentrid *Stegastes fuscus*, one of the most abundant species found in the Currais Archipelago and Itacolomis Island (Santos, 2014), has a short larval cycle and short larval dispersal range (Daros et al., 2016), indicating that for this species the probability of dispersal beyond their home reef is low (Grande et al., 2019).

The arrival of fish larvae on the appropriate habitat does not depend solely on physical dispersal processes, where tiny larvae simply go where currents take them. Many larvae use a range of sensory cues to detect, orient toward, and settle onto suitable reef habitats (Leis et al., 2002; Montgomery et al., 2006; Arvedlund and Kavanagh, 2009; Leis et al., 2011; Gordon et al., 2018). According to Leis et al. (2002), larvae can hear and distinguish a biologically generated sound from an ecologically meaningless, artificial sound. Chemical cues may also provide information to larvae when recognizing microhabitats for settlement (Atema et al., 2002; Kingsford et al., 2002), with these chemical cues associated with the presence of food (Batty and Hoyt, 1995; Kolkovski et al., 1997; Lecchini et al., 2011), competitors (Ben-Tzvi et al., 2010) or predators (Dixson et al., 2010). In this sense, it is possible that some fish larvae will not settle on ARs without a chemical or sound stimulus.

The absence of a given species from the samples does not necessarily indicate absence from the site itself. Markedly patchy distributions and rarity of larval fish (Leis, 1991; Murphy and Willis, 1996) naturally imply a low probability of capture. It is not unusual for reef fish larvae of even a common species to be absent from most samples (Leis, 1989). Other possibilities are that species can occur at a site but may not be present during a survey period or that a species present during the sampling time is not detected by the method employed (Martin et al., 2005). These two situations promote 'false-zero' sampling and may have occurred in the present work. In the first hypothesis, the sampling period may have occurred on a different time scale of movement and presence of the missing species (Tyre et al., 2003; Martin et al., 2005). Most reef fishes have seasonal spawning patterns (Erdman, 1977) with larval settlement occurring in periodic short cycles on rare occasions (Victor, 1991). The second scenario may have occurred due to biases in sampling methods. In the case of light traps, reef fish larvae are not always representative of the natural environment (Leis and Goldman, 1987) because the susceptibility of post-larvae to sampling gear depends on phototaxis, which, although common, is not characteristic of all species (Doherty, 1987; Choat, 1993; Thorrold, 1992; Grorud-Colvert and Sponaugle, 2009). Moreover, avoidance of larval fish capture by plankton nets is a major source of underestimation associated with zooplankton abundance measurements (Clutter and Anraku, 1968; Wiebe and Holland, 1968; Wiebe, 1971). Reef fish larvae, at the end of their pelagic stage, have a relatively strong swimming capability (Buri and Kawamura, 1983; Fisher, 2005), which increases their ability to avoid nets leading to an underestimation of abundance. Nevertheless, we expanded the total number of fish species registered in the study area with a new occurrence of eight species and three families, showing that use of two methods combined reduced some of the sampling bias.

In the present study, we report the first preliminary characterization of the larval fish assemblage on ARs deployed off the coast of Paraná and observe differences between this larval fish community and that of the surrounding unconsolidated substrate (UN) in the summer. The results confirm that ARs appear to be effective in attracting fish larvae (and may even facilitate settlement and propagation of exotic species, such as the blenny *O. punctatus*) and indicate that artificial structures can improve fish production. However, to avoid equivocally concluding that ARs enhance productivity, it would be necessary to carry out additional sampling approaches in the future, such as DNA genotyping, otolith chemistry, and regional scale hydrodynamic modeling to predict the source population of fish larvae and help understand the connectivity with surrounding habitats. In addition, studies on artificial reef communities would benefit from careful comparisons with natural reef systems, and continuous monitoring studies to test whether ARs are evolving from attractors to being a productive area for eggs and larvae fishes. In this regard, the present study is the first step toward future research and development of better guidelines for sustainable use and management of ARs and natural rocky habitats off the coast of Paraná.

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AUTHOR CONTRIBUTIONS

C.B.A.: Conceptualization; Methodology; Investigation; Writing – original draft; Writing - review & editing;

H.G.: Data curation; Formal Analysis; Visualization; Writing - original draft; Writing - review & editing;

C.A.P.N.: Data curation; Formal Analysis; Visualization; Writing - original draft; Writing - review & editing;

R.H.L.: Investigation; Resources; Writing – review & editing

F.P.B.: Conception, Funding acquisition, Resources; Supervision; Project administration; Writing - original draft; Writing – review & editing.

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