

# Population structure of the lancelet *Branchiostoma caribaeum* (Cephalochordata: Branchiostomidae) in the Baía de Guanabara, Rio de Janeiro, southeastern Brazil

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**ABSTRACT.** Population structure of the lancelet *Branchiostoma caribaeum* Sandevall, 1853 was studied in four surveys, corresponding to austral seasons, in a tropical bay, southeast of Brazil. Abundance was higher in the spring and was positively correlated to coarse sediments, limiting its occurrence to some sectors of the sampling area. Body length and biomass differed seasonally but not between sexes. Sexually mature individuals occurred in all seasons, suggesting continuous breeding that is typical of tropical species. Variation in the frequency of small specimens indicates temporal differences in the intensity of breeding. The body length of recruits differed from other population of lancelets and the small length which *B. caribaeum* attained sexual maturity in Guanabara Bay may be related to local environmental stress or the great availability of food.

**KEY WORDS.** Amphioxus; autoecology; macrobenthos; marine invertebrates; soft-bottoms; sublittoral.

**RESUMO. Estrutura populacional do anfioxo *Branchiostoma caribaeum* (Cephalochordata: Branchiostomidae) na Baía de Guanabara, sudeste do Brasil.** A estrutura populacional do anfioxo *Branchiostoma caribaeum* Sandevall, 1853 foi analisada em quatro campanhas abrangendo todas as estações do ano, na Baía de Guanabara, sudeste do Brasil. A abundância de indivíduos foi maior na primavera e positivamente correlacionada com sedimentos grossos, tendo sua distribuição limitada a alguns setores da área amostral. O tamanho corpóreo e a biomassa diferiram sazonalmente, mas não entre os sexos. Indivíduos sexualmente maduros ocorreram em todas as estações do ano, sugerindo uma reprodução contínua, típica de espécies tropicais. Variações na frequência de ocorrência de espécimes pequenos indicaram diferenças temporais na intensidade da reprodução. O tamanho corpóreo dos recrutas diferiu de populações de anfioxos de outros locais. O menor tamanho em que *B. caribaeum* atinge a maturidade sexual na Baía da Guanabara pode estar relacionada ao estresse ambiental ou à grande disponibilidade de alimento do local.

**PALAVRAS-CHAVE.** Anfioxos; auto-ecologia; invertebrados marinhos; macrobentos; sedimentos não-consolidados; sublittoral.

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Benthic species are key indicators of estuarine environmental status, responding predictably to many kinds of natural and human-induced disturbances (THOMPSON *et al.* 2003, VENTURINI *et al.* 2004). Polychaets, mollusks, crustaceans and nematodes are the most common animals in marine soft-bottoms. However, other groups, such as the lancelets (also named amphioxus), dominate numerically some bottoms, having a putative important role in the structure and function of those habitats (GOSSELCK 1975, LIGHT 1923, STOKES 1996). Their larvae could also reach high densities in the plankton during reproductive events (BOSCHUNG & SHAW 1988). Due to its gregariousness in the benthos system, the habitats where they occur are named "amphioxus sands".

Lancelets are endobenthic organisms that live in coarse sediments, from intertidal to sublittoral bottoms, feeding

mainly on plankton and microphytobenthos (BIGELOW & FARFANTE 1948, RIISGARD & SVANE 1999), being important food items for fishes and crustaceans (STOKES & HOLLAND 1996). It is possible that lancelets play a significant role in the transference of microbial production to higher trophic levels due to their feeding mode (RUPPERT *et al.* 2000).

Most lancelets have sub-tropical and tropical occurrence but most population and reproductive studies were conducted on temperate species (STOKES & HOLLAND 1996). *Branchiostoma caribaeum* Sandevall, 1853 (Branchiostomidae) is the only benthic lancelet species that occur in Brazilian waters (POSS & BOSCHUNG 1996) and very little is known about its occurrence. This work describes some aspects of the population structure and spatial distribution of *B. caribaeum* from Baía de Guanabara, comparing with populations worldwide.

## MATERIAL AND METHODS

### Study Area

Baía de Guanabara (BG), located at 23°50'S, 43°08'W, is a 384 km<sup>2</sup> eutrophic coastal bay in southeastern Brazil, impacted by the heavy discharge of both industrial and domestic waste from the Rio de Janeiro metropolitan area and its drainage basin. High inputs of heavy metals, petroleum hydrocarbons and toxic chemical compounds enter the bay daily, especially in the inner portion, accumulating in the bottom sediments (KJERFVE *et al.* 1997). However, these pollutants seem to be buried in anoxic sediments and therefore, unavailable to biological uptake (CARVALHO *et al.* 1992).

The bay experiences a large spatial and temporal variability of its water quality, mainly caused by circulation patterns and pollution foci (KJERFVE *et al.* 1997). Circulation is controlled by tides and winds, allowing water inflow from the ocean through the bottom layers. As described by KJERFVE *et al.* (2001), BG has a complex bathymetry with a 400 m wide central channel, which stretches from the mouth (1.6 km wide) more than 5 km into the bay, and is defined by the 30 m isobath. The channel rapidly becomes shallower further into the bay, being 1 m depth in the inner portion. Because of the intense mud sedimentation, the mean depth in the bay is only 5.7 m.

According to PARANHOS *et al.* (1995) and KJERFVE *et al.* (2001), the worst water quality of BG is indicated by average faecal coliform counts higher than 1,000 ml<sup>-1</sup> and by the average chlorophyll concentration exceeding 130 µg l<sup>-1</sup> in the inner bay, the most critical zone, in response to high nutrient loading. Sediments are not evenly distributed at the bottom, predominating mud at the inner areas and sand near the mouth (BAPTISTA-NETO *et al.* 2006). As a result of increasing pollution levels and poor circulation towards the inner region, the sediments in this area present oxygen depletion, becoming anoxic during the wet season (MENDES *et al.* 2007).

### Sampling design and laboratory analysis

Samplings were taken in four oceanographic surveys with three-month intervals, according to austral seasons, in fall and spring of 2000 and summer and winter of 2001. A triplicate sample was taken with a 0.1 m<sup>2</sup> van Veen grab in 38 sampling stations. The sampling stations were grouped into inner (stations 1-14), intermediary (stations 15-26), and outer sector (stations 27-38) in relation to natural hydrodynamic characteristics and pollution intensity established in previous work (MENDES *et al.* 2006, 2007) (Fig. 1).

Sediment samples were sieved out through a 1.0 mm mesh size and the lancelets sorted under stereomicroscope. Taxonomic determination was based on the revision work of POSS & BOSCHUNG (1996). The Frequency of Occurrence (FO) of the lancelets was calculated for each of the three sectors of the study area. Depending on FO values, the lancelets were classified as constant (F > 50%), common (10% < F < 50%) or rare (F

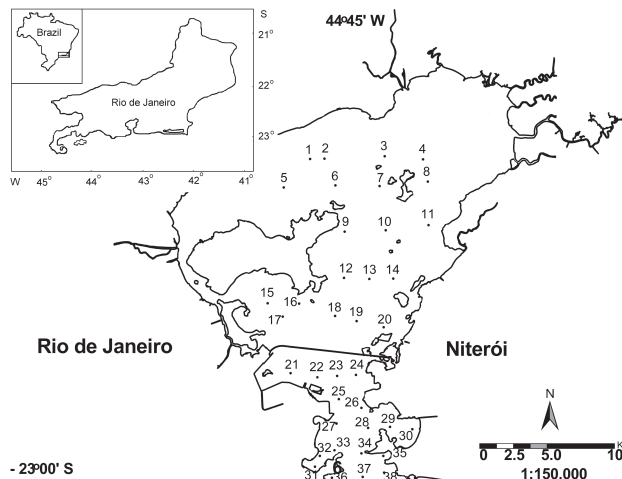


Figure 1. Geographic location and distribution of sampling stations at Baía de Guanabara.

< 10%). Density, wet weight biomass and the body length were determined for each sample.

Organisms were fixed in 4% formalin and preserved in 70% ethanol with glycerin. Because lancelets did not show external sexual dimorphism, sexes were determined by inspection of gonad colors under a stereomicroscope (white in males and orange or red in females). Samples were stored in dark to avoid loss of color. One side of the body wall was randomly choose to observe under stereomicroscope by transparency. A gonad index was used to infer the reproductive development of the population. The index used was the Proxy Gonad Index (PGI), proposed by STOKES & HOLLAND (1996) and calculated by the following formula:  $PGI = GI \cdot Gw \cdot Gh \cdot k / Bwt$ , where GI is the gonad length, Gw the gonad width, Gh the gonad height, Bwt the total body weight, and k the constant  $2.9 \times 10^{-6}$ . Specimens below 15 mm had no detectable gonads or very tiny ones and, therefore, sex and PGI could not be determined.

Statistical differences in body length and wet weight biomass among seasons were verified by non-parametric Kruskal-Wallis test. Chi-square test was employed to verify the hypothesis that sex-ratio is 1:1. The relationship between body length and wet weight biomass was analyzed by Pearson correlation coefficient and fitting to a polynomial regression.

Sediment samples were analyzed for grain size distribution, organic matter and carbonate content. The methods of mechanical dry sieving and decantation described by TANNER (1995) were used to determine the grain size fractions. The percentage of total organic matter was determined by loss of mass on ignition and sediment samples were oven-dried at 105°C for 12 hours and subsequently ashed at 500°C for four hours. Biodetritic carbonate (CaCO<sub>3</sub>) was obtained by HCl 10% attack. The relationship between lancelets density and sediment abiotic variables were determined by multivariate exploratory analysis. Normalized mean values of lancelets density, fine grain fraction, sand

grain fraction, gravel grain fraction, carbonate and organic matter content were organized in a data matrix to have similarities between stations calculated by Euclidean Distance. Similarity matrix was used to run a Multidimensional Scaling Analysis, using Primer 6 software and results were showed as ordination plots.

## RESULTS

*Branchiostoma caribaeum* in BG was a rare species in the inner sector while in the intermediary and outer sectors it was constant. Abundance was higher in spring and in the outer sector (Fig. 2). Considering the whole sampling design, the species showed a highly aggregated and patchy distribution reaching a maximum of 38 ind.  $0.1\text{m}^{-2}$ .

Body length ranged from 3.30 to 50.90 mm, with no statistical difference between sexes. Also, wet weight biomass was not statistically different between sexes, ranging from 0.0001 to 0.1284 g. Length and wet weight biomass differed statistically among seasons (KW = 30.9,  $p < 0.001$ ). A visual inspection of the length frequency distribution histogram showed the abundance of specimens below 15 cm (with no conspicuous gonad; presumably juveniles) was higher in the winter (Fig. 3). Length and wet weight biomass was highly positively correlated ( $R = 0.82$ ,  $p < 0.01$ ), showing a polynomial relationship (Fig. 4).

According to the Chi-square test results, sex-ratio did not depart statistically from 1:1. Sexually mature individuals, both male and female, occurred in all seasons. The majority of individuals bearing conspicuous gonads was between 25 and 35 mm long. The smallest mature male and female were 16.85 and 21.44 mm, respectively (Fig. 5). The PGI increased from fall to summer and decreased in winter (Fig. 6). Sexually mature individuals were found in all seasons but their relative occurrence was higher in winter (Fig. 7).

Ordination plots of lancelets' density and sediment abiotic variables showed that their occurrence and abundance in BG is positively related to coarse sediments (sand and gravel) and carbonate content, and negatively related to fine grains and organic matter content. Those plots also showed that lancelets are more abundant in the outer sector of the bay (Fig. 8).

## DISCUSSION

The occurrence of lancelets in BG is restricted to coarse sediments, as reported worldwide (WEBB 1955, BOSCHUNG & GUNTER 1962, GOSSELCK 1975, STOKES 1996, YAMAGUCHI & HENMI 2003, DOLBETH *et al.* 2006). It seems that sediment type is the main abiotic variable that drives lancelet distribution. In the inner sector of BG, where they have a rare occurrence, they were found in patches of coarse sediments. In the outer sector, where the hydrodynamics is more intense, coarse sediments are widespread, favoring the establishment of *B. caribaeum*. No experimental work has been done to elucidate that relationship with coarse sediments, which could be a collateral effect of the necessity of being located in areas of strong hydrodynamics to favor food availability.

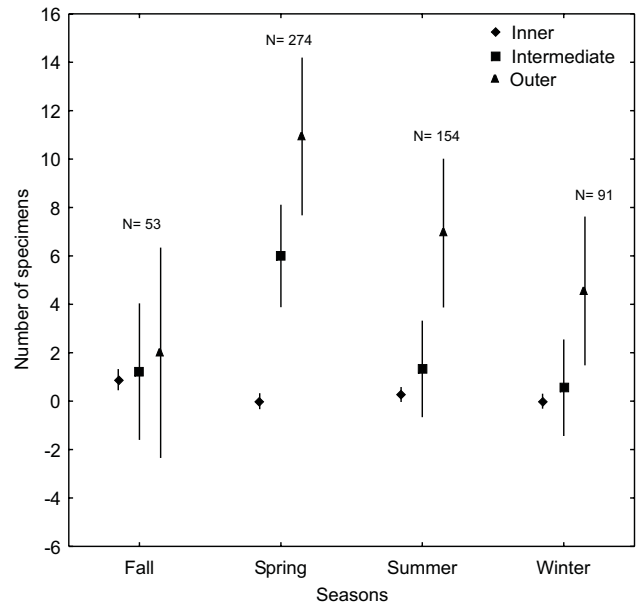


Figure 2. Mean abundance ( $\pm$  standard error) per  $0.1\text{m}^2$  of *B. caribaeum* in each sector and seasons at Baía de Guanabara. (N) Number of individuals sampled.

Lancelets in BG could reach high densities. Extrapolating the sampling area ( $0.1\text{m}^2$ ) to a square meter area, the maximum density found was about  $3,800\text{ind.m}^{-2}$ . This value is between the maximum densities reported to *B. floridae* Hubbs, 1922 in Florida –  $1,200\text{ind.m}^{-2}$  (STOKES 1996), and *B. senegalensis* Webb, 1955 in Spain –  $9,000\text{ind.m}^{-2}$  (GOSSELCK 1975). Being a filter-feeding animal, those high densities should be supported by the high concentrations of phytoplankton and bacteria in the water of BG (KJERFVE *et al.* 1997, PARANHOS *et al.* 1995). However, the sampling design adopted in this work did not allow recognition of the extension of that “amphioxus sand” in the bay to infer about the role of lancelets in the transference of pelagic production in the local food web, as suggested by RUPPERT *et al.* (2000). We suppose that such transference is stronger in the outer sector of the bay, where coarse sediments are widespread (BAPTISTA-NETO *et al.* 2006) and lancelets have a constant occurrence. Nevertheless, densities reported in the literature should be viewed with caution because they are extrapolations from areas of sampling devices. Lancelets could never reach those densities in nature since they have a patchy distribution and most sampling designs are not made to determine the extension of those patches.

Endogenous and exogenous factors have been related to reproduction. Among exogenous factors, experimental results by FUENTES (2004) showed that water temperature could control the breeding season of lancelets, and field works by STOKES (1996) and YAMAGUCHI & HENMI (2003) reported that *B. floridae* and *B. balcheri* GRAY, 1847 breed in warmer season. In BG, water temperature did not show a seasonal variation, raining being the most important environmental change in the bay between sum-

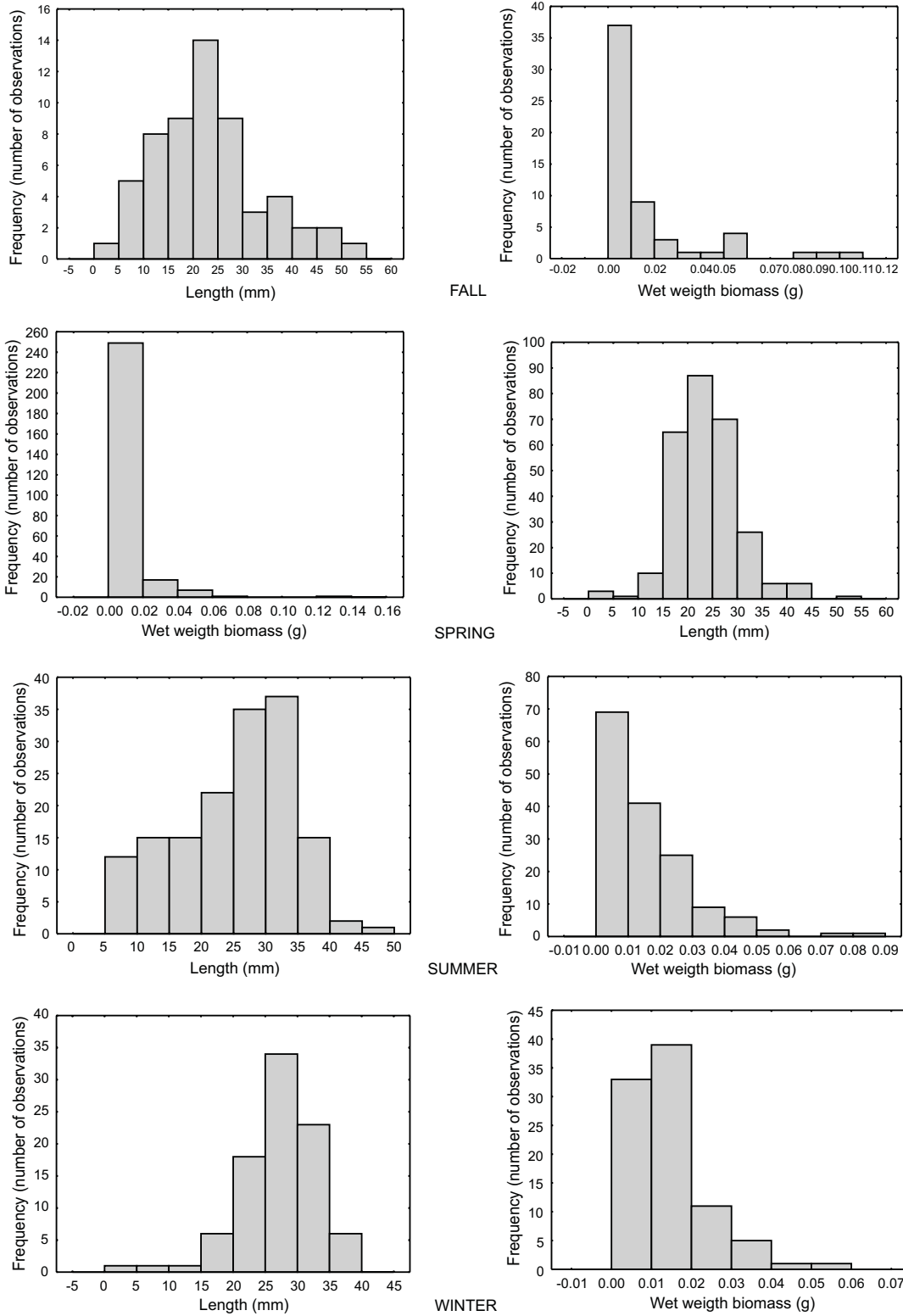
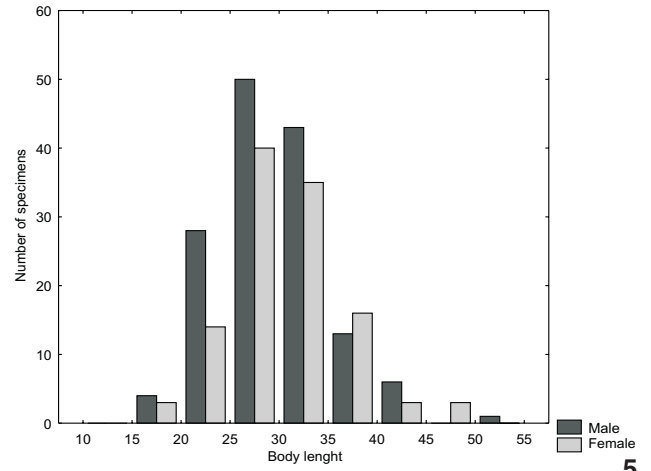
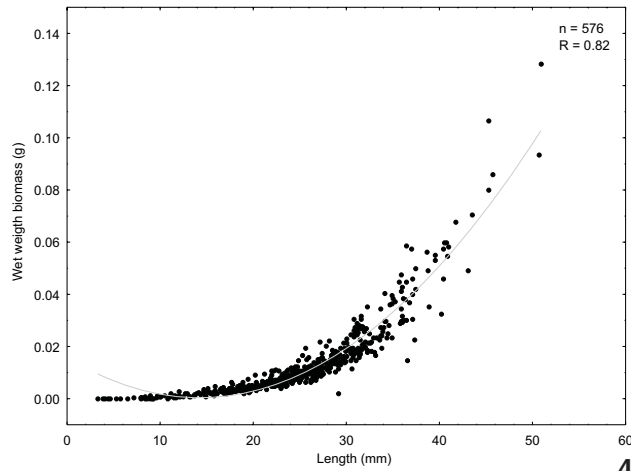


Figure 3. Frequency distribution of body length and wet weight biomass of *B. caribaeum* in each season at Baía de Guanabara.



Figures 4-5. (4) Relationship between body length and wet weight biomass of *B. caribaeum* from Baía de Guanabara. (R) Pearson linear correlation coefficient. (5) Body length frequency distribution of male and female specimens of *B. caribaeum* with matured gonads at Baía de Guanabara.

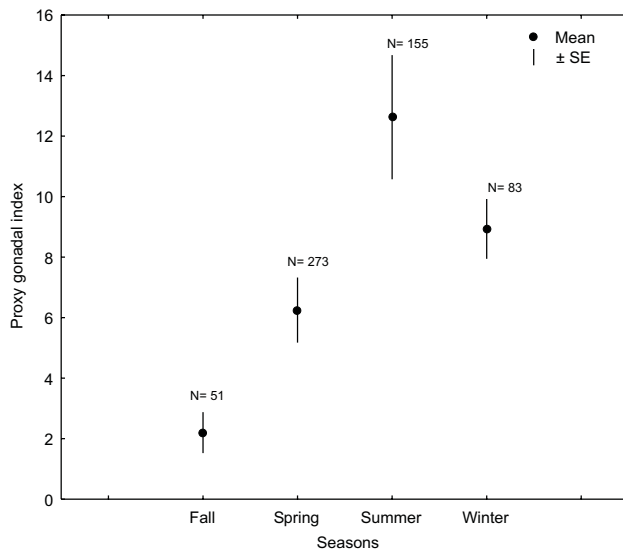


Figure 6. Proxy gonadal index of *B. caribaeum* in each season at Baía de Guanabara.

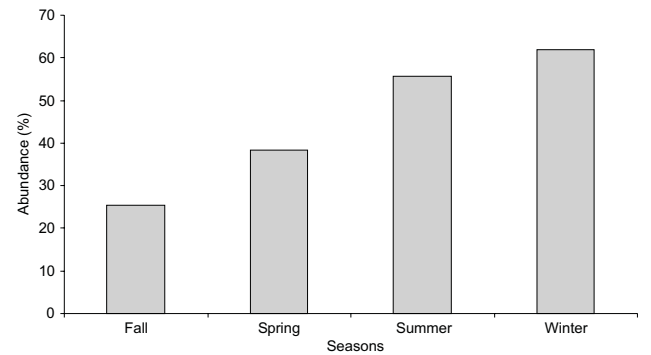


Figure 7. Percentual abundance of *B. caribaeum* mature specimens in each season at Baía de Guanabara.

mer (wet season) and winter (dry season). However, as a result of the small water residence time within the bay, salinity did not change expressively, except near the mouth of rivers (KJERFVE *et al.* 1997). In spite of the variations observed in the gonad index of *B. caribaeum* in BG, sexually mature individuals occurred in all seasons, suggesting a continuous breeding, typical of tropical species, although an expressive variation in the relative abundance of mature specimens was observed among seasons (from 25 to 60%, in fall and winter, respectively). The frequency of individuals in length categories also showed variations among seasons, the smallest classes being more abundant

in the summer. The size classes among 20 and 40 mm were the more abundant in all surveys while individuals greater than 40 mm were not found in the winter. Such variations could reflect differences in the intensity of breeding in the local lancelets population. In the northeast of Brazil (7°S), ALVES *et al.* (2001) also found sexually matured specimens occurring along the year but a different pattern of variation, the higher abundance of juveniles in winter and gonads more developed in summer.

The smallest body length is inferred as the length of recruitment (HENMI & YAMAGUCHI 2003, DOLBETH *et al.* 2006). According to that, the size *B. caribaeum* recruits in BG (3.3 mm) is quite similar to *B. floridae* (4 mm) from Tampa bay (STOKES 1996) but different of *B. balcheri* (7 mm) from Japan (HENMI & YAMAGUCHI 2003) and of *B. caribaeum* (7 mm) from the north-east of Brazil (ALVES *et al.* 2001). Differences in the minimum size could be related to sampling and sorting methods and in the maximum size to food availability, among other variables.

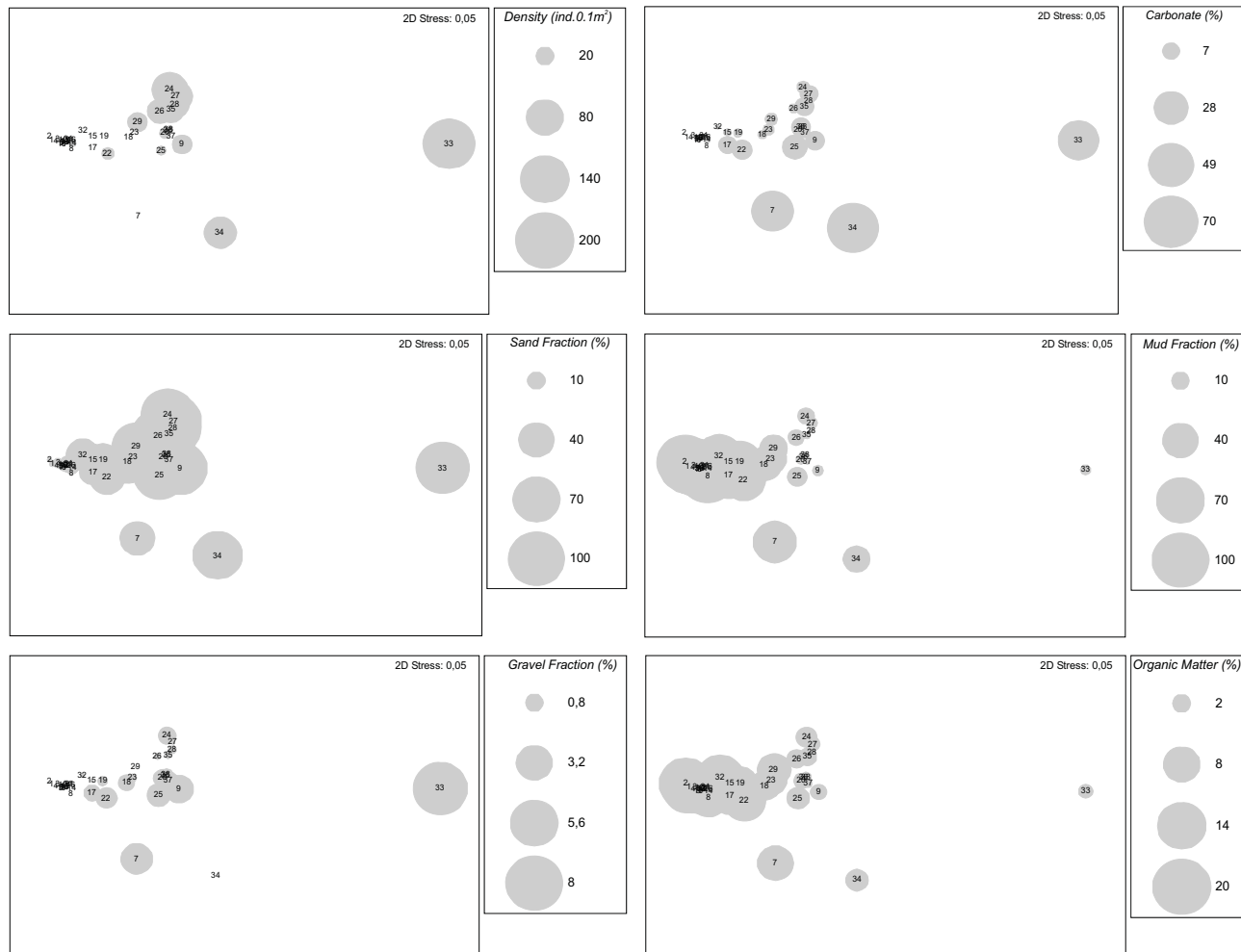


Figure 8. MDS ordination plots of densities of *B. caribaeum* (a); sand fraction (b); gravel fraction (c); carbonate content (d); mud fraction (e), and organic matter content (f) at Baía de Guanabara.

The size classes between 3.3 and 15 mm were less abundant in the winter indicating a less intense recruitment in that time.

Specimens below 15 mm had no detectable gonads indicating the size at the onset of sexual maturity. Values reported in the literature range from 18 to 40 mm for *B. floridae* and *B. lanceolatum*, respectively (STOKES 1996, DOLBETH *et al.* 2006). The smaller length which *B. caribaeum* attained sexual maturity in BG may be related to local environmental stress or to favorable feeding conditions that could lead to individuals maturing earlier (KJESBU 1994, LUCAS & LAWES 1998, YONEDA & WRIGHT 2005).

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