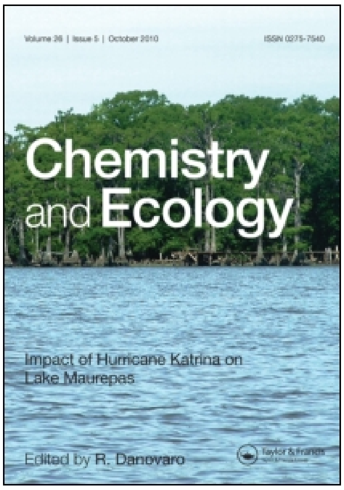

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RESEARCH ARTICLE

The European lancelet *Branchiostoma lanceolatum* (Pallas) as an indicator of environmental quality of Tuscan Archipelago (Western Mediterranean Sea)

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In June 2004 we found adults of *Branchiostoma lanceolatum* (Pallas, 1774; Cephalochordata) in sediment sampled within the integral reserve zone on the western side of Capraia island. The collecting site was located at 33 m depth in front of Punta del Fondo, by an extended *Posidonia* bed. The rippled substrate was dominated by coarse to very coarse sand (78.61%) while the silt-clay fraction accounted for less than 3%. The organic contamination analysis of the sediment gave results typical for unpolluted areas in the Mediterranean: total organic carbon 1.2%; 16 US-EPA PAHs 1.57 ng/g d.w., with a predominance of high molecular weight compounds; total PCBs 0.36 ng/g d.w., with a prevalence of higher chlorinated congeners; PBDE 16.26 pg/g d.w.; HCB and *pp'*-DDE below the limits of detection. Once abundant along the Italian coasts, *B. lanceolatum* is now rare and apparently confined to marine protected areas. In Tuscany, the species was known so far only from the Meloria Shoals. Most data in the literature suggest that its demise is related to the change in texture and eutrophication of the coastal sediments. Future studies should investigate the species' tolerance to chemical pollutants.

Keywords: Cephalochordata; amphioxus; Capraia; Tuscan Archipelago; seabed indicator; marine protected areas

1. Introduction

In 2004, while monitoring the contamination status of sediments in marine protected areas of the Tuscan Archipelago, we came across a few adult specimens of the European lancelet, *Branchiostoma lanceolatum* (Pallas, 1774), in samples from Capraia island [1].

Lancelets, also known as amphioxi, are slender, fish-like animals of inconspicuous appearance, only 5 cm long, which spend most of their time buried in marine sands. They are the sole living members of the subphylum Cephalochordata, phylum Chordata, which is comprised of some thirty species classified in three genera (*Branchiostoma, Asymmetron* and *Epigonichthys*) and distributed worldwide in temperate and tropical seas at depths of 0–230 m. Since the earliest studies, for their extraordinary anatomy, these animals appeared to have evolved on the borderline between invertebrates and vertebrates. Molecular biology has confirmed that, among all

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living protochordates (hemichordates, urochordates and cephalochordates), lancelets are those genetically closest to vertebrates, even though their genome has lower complexity and size [2].

Lancelets, both in their planktonic larval and benthic adult phases, are filter-feeders [3]. Adults, depending on the sediment grain-size, lie partly or fully buried in oblique position, with the buccal opening and metapleuric folds directed upwards. A better circulation of water, facilitating the incurrent flow through the mouth and the excurrent flow through the atrial pore, is thought to explain their general preference for coarse sandy substrates [4]. Cephalochordates are mostly associated with oxygenated sediments, but the recently discovered species *Asymmetron inferum* Nishikawa lives in anaerobic and sulphide-rich environments [5].

Adult lancelets are relatively sedentary. They seldom emerge spontaneously above the sediment, or only for very brief periods, except at spawning time. Nevertheless, the majority of species have wide geographic distributions, thanks to their long-lived larvae (up to four months) which act as a powerful dispersive phase. In particular conditions, larvae can disperse as far as 3000 km away from their parent population [6].

Natural populations of lancelets can locally attain very high densities. In Florida, *Branchiostoma floridae* Hubbs reaches 1200 individuals m^{-2}, and in Jamaica, the same species has scored densities of 5000 individuals m^{-2} [7]. In some parts of the world these animals have been used as an easy food source for humans (*Branchiostoma belcheri* Gray; China) [8] or for domestic animals reared on the beach (*Branchiostoma platae* Hubbs; Brazil) [9]. However, there is clear evidence that, due to various man-made disturbances of the marine environment, the overall population sizes have decreased [10]. For instance, in past years the granite sand bars southwest of Uryu island in Japan used to harbour one of the most abundant populations of cephalochordates (*B. belcheri*) worldwide. This species has been decimated as a consequence of gravel extraction which lasted in the area for more than 25 years, and resulted in the influx of mud into the sand bar. The habitat transformation not only brought about a major change in the benthic fauna, but in turn affected the fish community of the sand bar and also animals at higher trophic levels, such as the red-throated loon and the finnlessback porpoise [11]. *B. belcheri* is now ranked as vulnerable in the Japan Red Data Book of marine species, because of its general numerical decline. Also in other regions of the world, the lancelets are commonly described as a rare and threatened species.

The European lancelet, once very abundant along the northeastern Atlantic coasts and in the Mediterranean [12], has become increasingly rare since the first decades of the 1900s [13] and is also listed in the Black Sea Red Data Book [14]. The few recent records from the Italian coastline, including the present one, are all at current or planned marine protected areas, suggesting that the species finds refuge only in long-undisturbed spots of the sea bottom. Besides documenting a new record (which is the second ever for Tuscany), this paper gives the opportunity to call more attention to the demise of this phylogenetically relevant animal and its associated community, whose status and ecological significance may indeed need a more focused monitoring.

2. Materials and methods

Capraia island is located 42 km northwest of Elba in the Ligurian Sea, and is only 31 km away from Corsica. It is the westernmost of the seven islands forming the Tuscan Archipelago and is of volcanic origin, with a coast largely made up of steep rocky cliffs and only one beach (Cala Mortola), by the northern tip. The western side of the island is made of pyroclastic rocks and, because of its steepness, is crossed by many short torrents which split those rocks into a coarse detritus and discharge it directly into the sea; on the eastern side of Capraia, trachyandesites prevail, which are harder to erode, and marine sediments are finer [15]. A narrow bed of *Posidonia oceanica* surrounds the whole island, in so far as the rocky shores allow, and about 80% of the

Posidonia bed occurs along the western shore, where it can reach 40 m depth. The island is part of the European network 'Nature 2000' as a site of community importance, and is incorporated in the Italian National Park of the Tuscan Archipelago (D.P.R. 22.7.1996) and in the International Pelagos Sanctuary for Mediterranean Marine Mammals (1999 Barcelona Convention). The marine protected area of Capraia surrounds the whole island except for a free zone at the northeastern corner, in front of the port and the town. The highest level of protection (Zone A) is on the midwestern side, between Punta della Manza and Punta del Trattoio, where human access, diving, fishing and navigation are not allowed. Zone B, which comprises the rest of the MPA, allows some forms of recreational and commercial fishing, though commercial trawling is banned here, as it is everywhere else in the Tuscan Archipelago National Park.

On 16 June 2004, with permission from the park management, the sea bottom at 33 m depth in front of Punta del Fondo (Figure 1a, b, c), included in Zone A, was explored by scuba diving by the second author. Buckets of sediment were manually scooped near an extended *Posidonia* bed at 43°02.389' N, 09°47.589' E. The rippled coarse-sand substrate reflects the strong currents in the area (Figure 1c). The sediment comprised a dominant (90%) red-black mineral component, derived from the weathering of volcanic rocks and pyroclastic deposits, and a little organogenic detritus (fragments of echinoderm spines, bryozoan skeletons and mollusc shells) (Figure 1d). The organic fraction was scarce, mostly represented by seagrass debris. The sample for faunistic analysis was fixed in 7% formalin. The whole zoobenthic community was extracted in the laboratory using elutriation and filtering on a sieve with 0.15 mm mesh size; it was then sorted under a stereomicroscope, and transferred to 70% alcohol.

The sediment samples for organic contamination analysis were freeze-dried and extracted using an ultrasonic bath system. PAHs (polycyclic aromatic hydrocarbons), PCBs (polychlorinated

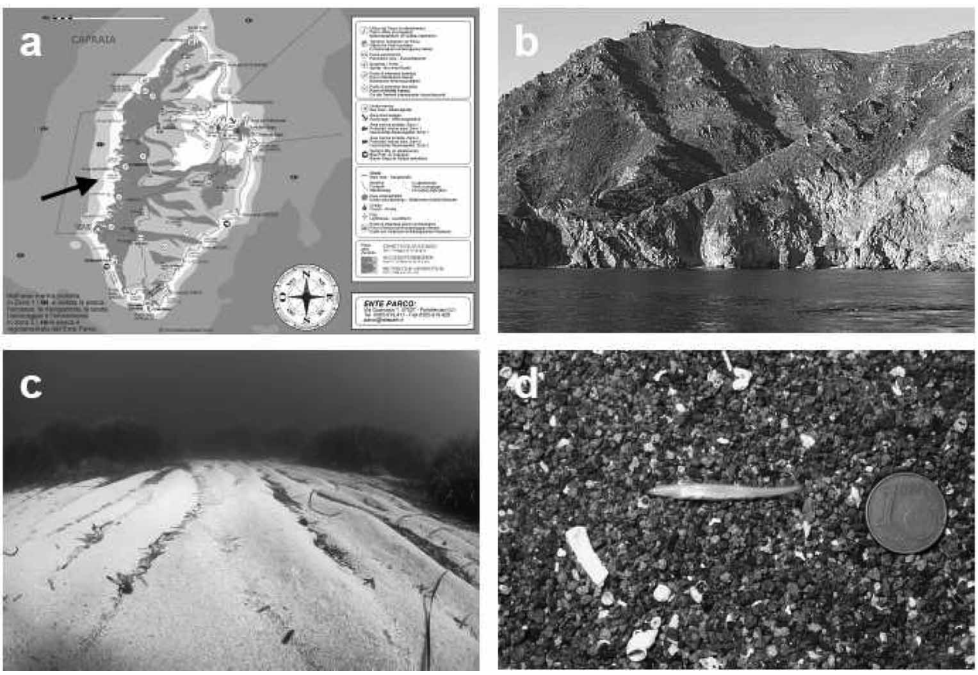

Figure 1. The collecting site of *Branchiostoma lanceolatum* at Capraia. (a) Map of the island (courtesy of the Tuscan Archipelago National Park). The arrow points to the sampling area on the western side. (b) A view of Punta del Fondo from 2 km offshore. (c) Location of the finding at 33 m depth, near an extended *Posidonia* bed. (d) *B. lanceolatum* (preserved female) and its coarse sandy substrate at Capraia (with a 1 euro cent for scale).

Table 1. Concentrations (mean ± standard deviation) of organic contaminants and total organic content in surface sediments from the Punta del Fondo sampling station.

Σ16PAH ng/g d.w.	Σ PCB ng/g d.w.	HCB ng/g d.w.	*pp'*-DDE ng/g d.w.	PBDE pg/g d.w.	TOC (%)
1.57 ± 0.2	0.36 ± 0.33	< 0.1	< 0.1	16.26 ± 4.22	1.2

biphenyls), *pp'*-DDE (para-para-dichlorophenyl-dichloroethylene), HCB (hexachlorobenzene), and PBDE (polybrominated diphenyl ether) were determined and quantified using GC-ECD (gas chromatography – electron capture detector), GC-MS (gas chromatography – mass spectrometry) and HPLC (high performance liquid chromatography).

Grain-size composition was determined using a set of steel test sieves (DIN EN ISO 9001) of various diameters; collected data for each size class were expressed as percentage dry weight. TOC analyses were performed according to the titration Walkley-Black method modified by [16]; results were expressed as percentages.

3. Results

The count of myotomes confirmed the identity of the lancelet specimens (Figure 1d) as *B. lanceolatum*, which is recognisable by the following myotome formula: 36.1 (34–38) preatriopore + 13.8 (11–17) atriopore to anus + 11 (10–14) postanal [17]. The species appears to be abundant at Capraia: 690 cc of sediment contained three mature females. In the same sample was a diverse assemblage of Platyhelminthes, Nemertina, Nematoda, Crustacea, Acari, Polychaeta, Oligochaeta (tubificoid naidids and enchytraeids), Gastropoda, Bivalvia, Scaphopoda, Echinoidea, Ophiuroidea and Chaetognatha.

The seabed at the sampling station is characterised by coarse-grained sediment. The dominant fraction (58.81% of the total mass) is the very coarse sand (1000–2000 μm), followed by the coarse sand (500–1000 μm) with 19.8%. The gravel fraction (>2000 μm) takes up 14.65%, while the fine to medium sand fraction (63–500 μm) takes up 3.87%. The silt-clay fraction accounts for less than 3%. We observed that sediments are characterised by low levels of silts throughout the Capraia integral reserve zone.

Table 1 shows a summary of the chemical analyses of the sediment. Among the 16 US-EPA PAHs, fluoranthene (Flu, 26%), pyrene (Py, 14%), benzo(a)anthracene (BaA, 11%) and chrysene (Chry, 11%) represented over 60% of the total PAHs, indicating a predominance of high molecular weight PAHs in respect to low molecular weight ones. The amount of carcinogenic PAHs (e.g. benzo(a)pyrene) was low. The identified PCB congeners were predominantly higher chlorinated biphenyls, i.e. hexa- to octachlorobiphenyls, which can be explained by the persistence of this sort of pollutants. The concentrations of HCB and *pp'*-DDE were below the limit of detection (<0.01 ng/g) for all sediment samples. PBDE were low and this is indicative of a relatively unpolluted environment. The total organic carbon content was 1.2%.

4. Discussion

4.1. *Historical and recent distribution records in Italy*

The European lancelet was first discovered in 1774 on the Cornwall coast of England and erroneously classified among the nudibranch gastropod molluscs (*Limax lanceolatus*

Pallas, 1774) [18]. Sixty years later, Oronzo Gabriele Costa [19] re-discovered it in Naples (Cape Posillipo) and, recognising its affinities with lampreys and hagfishes, renamed it *Branchiostoma lubricum*. Unaware of this, Yarrell [20] studied it again in England; he too considered it related to the cyclostome fishes and changed its name to *Amphioxus lanceolatus*. Although the name *Amphioxus* has been much more successful, the rules of zoological nomenclature give priority and validity to the scientific name *Branchiostoma lanceolatum*.

B. lanceolatum could be found in the past along the entire coast of Europe and 'most abundantly in the waters of the Mediterranean sea, near Naples and Messina' [12]. In the Autumn of 1842, the German anatomist-physiologist Johannes Müller travelled with his family to Italy and wrote to his Swedish colleague Anders Retzius that in Naples *Amphioxus* occurred in huge amounts and could be easily caught while one was swimming [21]. At that time, this little fish lacking a head and backbone was considered a delicacy also in Italy [22]; it is thus no surprise that its popular names ('vermidduzzu i mari', 'zumpariello', 'murosa') still survive in the dialects of Sicily, Campania and Romagna. The hundreds of specimens conserved at the Zoological Museum "La Specola" of Florence University [23] bear witness to the density of the populations inhabiting the Gulf of Naples and the Strait of Messina at the end of 1800s. Already in 1951, however, Rheinard Dohrn, then director of the Zoological Station in Naples, complained about the local decline of the species to 'small numbers from time to time', and about its disappearance from most European waters [13]. Indeed, adults are reported today from Italy only in correspondence with existing or planned protected areas (for example, Cinque Terre, Meloria Shoals, Ischia, Cyclops Islands, Vendicari, Capo Gallo and Isola delle Femmine, Tremiti Islands, Miramare), where evidently lancelets find refuge in habitats not impacted by human activities. Some may suggest that the decline of the amphioxus is only apparent, the paucity of published records being due to a lack of specialists or the inadequacy of sampling techniques, or the fact that species inventories are rarely published today as they are considered too basic or 'not scientifically relevant'. To this, however, we object that (i) the amphioxus is a very characteristic animal, easy to identify; (ii) its local populations can be very dense, which render the animals easily collectable; and (iii) the occurrence of amphioxus is something worth showing off (a 'showpiece'), being notoriously associated with a specific biological status of the seabed [24]. Thus, a lack of records during surveys involving presumably suitable habitats must be taken for real.

Capraia is, so far, the only island of the Tuscan Archipelago where *B. lanceolatum* has been recorded. The species also occurs at the Meloria Shoals [25,26], some 60 km to the northeast, but no other record is known from Tuscan waters. Nor is there any historical record from Tuscany in the collections of the Florence Museum 'La Specola' [23]. Records of *B. lanceolatum* in the Ligurian Sea appear more frequent along the coast of Liguria, e.g. in Nature 2000 sites such as Fondali Nervi Sori (Genoa), Fondali di Punta Levanto and Fondali di Punta Mesco-Rio Maggiore (La Spezia) (source: http://www.natura2000liguria.it, May 2007). Below Tuscany, the next records of *B. lanceolatum* are from southern Latium and Campania and further south, while there seems to be a gap of the species range in the northern Tyrrhenian Sea. The Capraia population happens to be the southernmost in the Ligurian Basin and it thus may be of both conservation and biogeographic interest, being located at the northern end of the Corsica channel, a one-way gate from the Tyrrhenian Sea towards the Ligurian basin [27].

4.2. *Habitat characteristics and conservation requirements*

The typical habitat for *B. lanceolatum* is sublittoral coarse grained sediments in high energy environments ('Amphioxus sands'). Interestingly, the activity in sand of *B. lanceolatum* has been shown to cause *per se* a sorting of the substrate material, so that large grains come to rest at the surface with the finer material beneath [28]. In seas swept by strong tides, like the English Channel, Amphioxus sands can occupy large areas, even at some depth (circalittoral zone) [29]. In the Mediterranean, which is almost tideless, this type of habitat is mainly found in infralittoral deposits exposed to bottom currents ('Sables grossiers et fins Graviers sous l'influence des Courants de Fond'; SGCF) [30]. Allochthonous organogenic components, originating from adjacent *Posidonia* beds and/or littoral rock communities and transported there by the currents, may constitute an important substrate fraction. The SGCF biocoenosis, which includes a rich and diverse interstitial microfauna, can also develop inside the *Posidonia* meadows, along the intermatte channels, where bottom currents impede significant deposits of vegetal debris [30].

Amphioxus sands as described above seem to be rather frequent around Capraia, where, in places, they extend seaward down to 100–120 m depths [31,32]. Amphioxus sands are also reported for Elba and Giglio Islands [33] but no previous study has ever recorded *B. lanceolatum* in the Tuscan Archipelago, suggesting that the species is rare and localised.

Indeed, several species of other animal groups known as typically associated with the amphioxus, including some marine oligochaetes [34], have become rare or extinct in many historical localities. The reasons for decline/extinction are certainly related to prolonged anthropogenic disturbances of our coastal marine environment (trawl and dredge fishing, sand and gravel extraction, construction of piers or other shorezone structures, runoff from polluted rivers and sewage outflow), which have caused habitat loss or degradation *via* permanent changes of the coastal hydrodynamism, input or re-suspension of fine particulates, and eutrophication or pollution of the proximal coastal zone. Desprez [35] has shown that a long-term dredging of sand and gravel from a site on the French coast of the Channel, induced a change of the benthic community structure from one of coarse sands with *Branchiostoma* to one of fine sands dominated by polychaetes (*Ophelia borealis*, *Nephtys cirrosa*, and *Spiophanes bombyx*). In the sites of maximum impact, the alteration of the sediment dynamics caused an 80% reduction of species richness and a 90% reduction of abundance and biomass. In Europe, *B. lanceolatum* has been proposed as a 'seabed indicator' to support implementation of the EU Habitats and Water Framework Directives [36], in that it is intolerant of alterations such as substrate removal.

It should be stressed that disturbances created by dredging have longer-lasting effects in low-dynamic systems such as those prevailing in the Mediterranean, especially where conditions for regular redistribution of sediment do not exist [37]. In 1999, *B. lanceolatum* was recorded at a 44 m depth site off Capo d'Anzio (50 km south of Rome), during a pilot study for the evaluation of the environmental impact related to sand extraction for beach replenishment [38]. The amphioxus site was initially characterised by gravel 9%, coarse to very coarse sand 52.36%, fine to medium sand 35.91%, silt and clay 2.78%; total PCBs 0.37 ng/g d.w.; all organochlorine compounds <0.05 ng/g d.w.; low levels of Cd, Cu, Hg, Ni, Pb and Zn; low organic matter content (1.6%). Although external to the extraction area, the site underwent changes of the sediment composition: the gravel fraction decreased to 2.5%, and the silt-clay fraction increased to 12.8%. *B. lanceolatum* was not recovered during the post-dredging monitoring, nor even one year after cessation of the dredging activities (which had lasted less than five months). More generally, the former benthic community, typical of coastal detritic bottoms, had been replaced by one typical of coastal terrigenous muds [39].

Changes in granulometry are important in controlling the benthic distribution of lancelets but may be not the only factor. It is worth noting that, one century ago, *B. lanceolatum* could be found both in coarse and fine sand at Posillipo, Naples, even though the animals inhabiting fine sand had a smaller body size [40]. According to [41], it is sediment permeability that ultimately affects lancelet distributions. Konsulova [42] attributed the dramatic reduction of *B. lanceolatum* along the Bulgarian Black Sea coast to the expansion of muddy areas following the intensive dumping of organic waste. A higher organic content incorporated in the sand matrix may have strongly decreased the sediment permeability by clogging the pore water space.

Little is known of the tolerance of the species to chemical pollution, although in current benthic quality indexes (e.g. AMBI [43], BENTIX [44]) *B. lanceolatum* is classified among the species sensitive to organic enrichment and present under unpolluted conditions. Levels of organic contaminants in the sediment at the Capraia site are those typical of non polluted areas in the Mediterranean. The grain size composition of the sediment may play an important role in reducing the site contamination, in view of the smaller adsorption capacity of coarse sands as compared to fine sediment particles [45]. Capraia island sediments are characterised by low levels of silts throughout the integral reserve zone, probably due to the remoteness of both terrestrial and anthropogenic sources of fine particulates in the sampled areas. However, the presence of trace concentrations in all samples demonstrates that chemical substances originating from human activities are capable of reaching the totally protected zone and thus should be monitored to ensure that future problems do not develop.

4.3. *Ecological significance and concluding remarks*

We hope our paper will foster an interest in lancelets and their conservation. To assemble reliable faunistic and ecological information on these animals is not only important for conservation issues (to establish and monitor the distribution and status), but also because lancelets often play important roles in subtidal sandy ecosystems. For instance, *B. lanceolatum* is a key element in the diet of the Portuguese sole *Synaptura lusitanica* [46].

Ueda and Sakaki [47] even suggest that dense lancelet populations can play a role similar to that of earthworms in soil, because through bioturbation they can help the activity of seawater purification of sandbanks by sorting sands and maintaining DO concentration in sediment at a higher level, thus enhancing decomposition of organic matters by aerobic bacteria.

Future reports should provide detailed information on the sites' characteristics, including the chemical status of the sediments, in order to better qualify *B. lanceolatum* as a prospective macrobenthic indicator (see [48] for *Branchiostoma floridae*). A faunistic program addressing the whole amphioxus community would provide an important contribution to the management of the study areas, but also useful tools for assessing and monitoring the effects of climatic and environmental changes occurring in the Mediterranean. Last, a genetic comparison between the recorded populations could indicate whether the present range of distribution of *B. lanceolatum* in the Mediterranean is fragmented or it is made continuous by the prolonged larval stage and their effective spreading in the plankton.

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